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THE GASTRIC CÆCA AND THE CÆCAL BACTERIA OF THE HETEROPTERA.

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Summary.

1. Certain of the Heteroptera are almost unique among insects in the possession of well-developed cæcal appendages situated at the extreme posterior end of the digestive portion of the gut.

2. The cæca are remarkable in that wherever they occur they are invariably filled with bacteria.

3. These bacteria are not only always present in the cæca of normal bugs but the association is hereditary, the organisms appearing early in the alimentary canal of the developing embryo.

4. Structurally the bacteria from different hosts vary greatly, ranging from minute, coccus-like bacilli measuring often less than one micron, to huge, spirochæte-like forms thirty microns and more in length; but in whatever form they occur they are morphologically characteristic for the particular species harboring them.

5. That these are unquestionably bacteria is shown conclusively by culture experiments checked by agglutination tests.

6. These normal bacteria appear not only to inhibit the development of foreign bacteria but to exclude them altogether,

the mid-intestine being normally wholly free from the invading bacteria and protozoa which are common in many related insects· and this is probably the chief function in the life processess of the host performed by these cæcal bacteria.

7. The cæcal appendages themselves appear to be of profound phylogenetic significance, showing a complete gradation from extremely simple to very complex forms and in many cases indicate relationships contrary to those often assumed in the arrangement of the groups.

INTRODUCTION.

In certain groups of Heteroptera the alimentary canal is characterized by the presence of peculiar sac-like appendages which open into the mid-intestine at its extreme posterior end. These structures vary greatly in form and in degree of development in the different families in which they occur, but all have essentially the same histological structure and all agree in the fact that they invariably contain great masses of bacteria, apparently in pure culture, which are morphologically characteristic for the families and often for the genera in which they occur.

These structures were noted as early as 1809 by Treviranus in a pentatomid, *Pentatoma rufipes* (*Cimex rufipes*), and again in 1811 by Ramdohr in *Pyrrhocoris apterus* as well as in representatives of the Pentatomidæ, and they were subsequently observed and studied by numerous other investigators, notably by Dufour in 1833. It was not until about 1888, however, that Professor S. A. Forbes (Forbes, '96), in the course of an elaborate series of investigations on the contagious diseases of insects, discovered the remarkable association existing between certain Heteroptera and the bacteria which he found uniformly inhabiting the so-called cæcal glands. Leydig had previously noted the presence of these organisms in the cæcal appendages of a pentatomid in 1857, but he did not suggest their true nature and merely expressed surprise at the occurrence of such unusual structures in these organs.

The cæcal bacteria were really first observed by Professor Forbes in 1882 in crushed specimens of the chinch bug, *Blissus leucopterus*, and a brief technical description of the organism, under the name *Micrococcus insectorum*, was given it by Professor

T. J. Burrill in 1883. The specimens first examined by Professor Forbes were from experimental cages of potted corn in which the insects had been dying in large numbers from some cause which even at the present time is not clearly understood, and upon finding large numbers of specific bacteria in crushed preparations of these insects he at first very naturally regarded them as the probable cause of the trouble, and proceeded to investigate the matter with characteristic thoroughness, with a view to the possible utilization of this organism as a means of controlling the chinch bug in the field. After a careful study, however, he was finally forced to the conclusion that the bacteria were not parasitic upon the chinch bug at all, but that they were really normal to the cæcal appendages of healthy bugs and that they probably had some important function in the metabolism of the insect. He also established later, by the examination of a great variety of insects of different orders, and especially of Heteroptera, that the chinch bug was not unique in this regard, but that the same phenomenon also occurred in a number of other species of Lygæidæ as well as in representatives of several other families of Heteroptera, and that wherever the cæca were present in this group, they were always filled with specific bacteria. A large part of this work was never published and the unpublished notes, which proved to be of the greatest assistance in this work, were turned over to me by Professor Forbes for use in continuing a study of the subject.

The problem as it was suggested to me by Professor Forbes was primarily to determine the significance of the presence of these "normal" bacteria of the cæca—to work out, if possible, the relation existing between the insects and their characteristic intestinal flora, and also, indirectly, to determine whether the occurrence of these complex cæcal structures might not throw some light on the phylogeny of the Heteroptera.

The following study was carried on under the supervision of Professor Forbes, and I consider it an honor to be in a position to express my indebtedness to him for his constant aid and encouragement throughout the course of the work.

I am also greatly indebted to Dr. W. J. MacNeal, in whose laboratory I was very kindly permitted to do a considerable

part of the work on the bacteriology of the subject, although at that time the laboratory was so crowded that few men would have thought of admitting me.

Constancy of the Cæcal Infection in Heteroptera.

One of the first questions that came up in connection with the work was that of the constancy of the bacterial infection of the cæca—whether these organs were always infected and whether the infecting organism was always the same. It had been established by Professor Forbes that the infection in *Blissus leucopterus* was constant throughout the range of this insect in Illinois, and all the Heteroptera in the neighborhood of Urbana, in which the cæca were present, also showed the infection; but to make certain that this relation was one of fundamental significance and that the infection was not merely a local phenomenon, peculiar to the Heteroptera of Urbana, or perhaps of Illinois, it was planned to select some common, widely distributed species and to examine specimens from as great a range as possible.

It was necessary, of course, that the species selected for a study of this kind should be widely distributed as well as common and easy to collect and, what was of even greater importance, that the cæcal bacteria should be characteristic in form, to insure that the infection was not due to different species of bacteria of similar form occurring in different parts of the range of the insect.

The harlequin cabbage bug, *Murgantia histrionica*, was finally chosen as the species most nearly fulfilling these conditions. It ranges from California across the southern half of the United States and thence to New England; and it is usually a very common and conspicuous form wherever cabbage is grown; but what made it especially suitable for this test was the large size and remarkable form of its cæcal bacteria (Plate VIII., Fig. 26). Instead of being minute, short rods, as in *Anasa tristis* and *Blissus leucopterus*, the only two other species available for the test, those of *Murgantia* are very large, contorted, spirochæte-like forms which could not possibly be mistaken for any of the other varieties of cæcal bacteria.

To secure specimens, requests for living material were sent to

a number of station entomologists located in parts of the country from which *Murgantia* had been reported. Living specimens were thus secured from ten states, extending in a continuous series along the southern and eastern part of the United States for a distance of between two and three thousand miles, including California, New Mexico, Oklahoma, Arkansas, Mississippi, Alabama, Georgia, South Carolina, North Carolina, and Maryland. The specimens of *Murgantia* for this study were obtained through the kindness of the following entomologists—the locations given showing the place where the insects were collected:

T. B. Symons, College Park, Maryland.
Franklin Sherman, Jr., Greenboro and Raleigh, North Carolina.
A. F. Conradi, Clemson College, South Carolina.
H. P. Stuckey, Experiment, Georgia.
W. E. Hinds, Auburn, Alabama.
R. H. Harned, Agricultural College, Mississippi.
Paul Hayhurst, Lanske, Arkansas.
C. E. Sanborn, Stillwater, Oklahoma.
Fabian Garcia, Agricultural College, New Mexico.
C. W. Woodworth, Live Oak, California.

In addition to these localities, specimens of *Murgantia* were also examined from different parts of Illinois, especially from the southern part of the state, where they were collected by Mr. L. M. Smith, of the state entomologist's office; and two specimens were also taken as far north as Urbana, Illinois.

Upon a comparison of the contents of the cæca of specimens from these widely separated localities, it soon became evident that the peculiar organism, first observed in the cæca of the two specimens collected at Urbana, was a constant inhabitant of the cæca of *Murgantia*, and that whether the individual examined was from California or Maryland this was invariably the highly characteristic, contorted organism first observed in the Urbana specimens.

While the cæcal organisms from all these localities are clearly of the same species, all having precisely the same typical form, there are certain occasional differences in size that it may be well to mention. It was observed, for example, that in the insects from Georgia, as well as in occasional specimens from

Illinois, the bacteria, although characteristic in shape, often averaged decidedly longer and stouter than those from most of the other localities; but this difference in size was not constant in all the specimens of *Murgantia* from these states, and it was doubtless due to some slight difference in the metabolism of the particular individuals examined.

Murgantia is the only species that I have studied in any numbers with this idea in mind, from outside Illinois; but hundreds of specimens of other species have been examined from different parts of this state, and all of these show, without exception, that in their morphology the type of cæcal organism is absolutely constant for a given host species.

Origin of the Cæcal Infection in the Heteroptera.

At an early stage in the work it became evident that one of the first things to be determined was the exact time and manner in which the infection with the cæcal bacteria normally takes place. Assuming that the normal bacteria would develop readily in artificial cultures, the next essential step would be to differentiate between this organism and any of the common saprophytic or parasitic forms that are so frequently met with in the alimentary canal of most insects, and which would naturally be expected to appear also in cultures from the cæca. Until the identity of the normal species in culture could be established beyond question, it would clearly be useless to undertake any time-consuming study of the physiology of any of the organisms that might be isolated in an attempt to correlate this with the digestive processes in the insect; and at the commencement of the work it appeared that the only possibility of certainly differentiating between the normal bacteria and the contaminating forms that might appear, was in some way to secure insects free from the infection, and then by direct feeding experiments to determine which were really the "cæcal" bacteria.

The normal bacteria are present, not only in the cæca, but also in considerable numbers free in the alimentary canal of the host and, as might be expected, they also occur in the brownish, liquid excrement of the insect. It is easy to imagine from this how the surroundings might be very highly contaminated with

this organism, especially in the case of many of the common gregarious, phytophagous Heteroptera, such as the chinch bug, the squash bug, or *Murgantia*. It is a very common habit with these insects, as I have frequently observed, especially when in the immature condition, to sample with their beaks any drop of liquid that they may find; such as drops of dew, drops of sap exuding from wounds in the host plant, or even the liquid excrement from other individuals.

From these facts, and since the bacteria appeared to be limited to the alimentary canal of the host, it was assumed that the infection probably occurred by way of the mouth and that the alimentary canal was doubtless invaded at some time during the early nymphal life of the insect.

Proceeding on this assumption, it was planned to rear the insects from the egg in sterile cages in order to have material for use in infection experiments with the different organisms that might be isolated from the cæca. *Anasa tristis* was selected at first for this rearing work, since the eggs of this species are very easily obtained in quantities at any time during the summer, and also because they are perfectly smooth and readily sterilized. As it was planned at first, the rearing of these sterile bugs should have been a comparatively easy matter. Since it was found that this insect is able to live and develop fully as well on the squash itself as upon the sap of the vine and leaves, the only essential equipment for a breeding cage would have been very simple indeed. The breeding cage, as I had intended to arrange it, was to have been merely a rather wide-necked Ehrlenmeyer flask of something like 500 c.c. capacity, plugged with cotton and sterilized. Small pieces of squash, removed aseptically, were then to be introduced into the flask, together with the sterilized eggs of the squash bug.

As a preliminary to this breeding work, experiments were carried out to determine whether aseptic material could be readily secured from the squash, and especially whether the eggs of the squash bug could be sterilized effectively without interfering with the subsequent development of the embryo. It was found that with a little care, pieces could be removed from a squash without any risk of contamination; and it was also

found that the development of the eggs of the squash bug was in no way hindered by a very thorough treatment with a solution of mercuric chloride. When such eggs were placed in bouillon as a check on the thoroughness of the disinfection, they would remain apparently sterile for a period of three days or more and then, at about the time the experiment should have been considered a success, an abundant growth would suddenly appear which apparently consisted of a number of different contaminating organisms. This test was repeated a number of times, with certain variations in the method of disinfection. It was thought at first that perhaps the film of air surrounding the egg prevented the aqueous bichloride solution from doing effective work, and the eggs were accordingly first moistened with alcohol to remove the film of air; but no matter how thorough the disinfection was made, the confusing growth almost always appeared.

Since there could be no question but that the surface of the eggs had been completely sterilized, there remained nothing but to conclude that the contaminating organism or organisms were within the egg itself.

As is well known, the eggs of birds may sometimes contain bacteria while still in the oviduct, and before the shell has been laid down, so that when the shell is formed they are included within it. There is no reason why the egg of an insect should not become contaminated in much the same way, and it was thought at first that this must be the case with the eggs of the squash bug. This species was accordingly abandoned, since it was clearly out of the question to rear sterile individuals of it. A little later in the season a few eggs were secured from specimens of *Murgantia histrionica* that had been received from Dr. W. E. Hinds, of Auburn, Alabama; and it was decided, as a last resort, to test these to see if they were also contaminated. These eggs were given exactly the same treatment as had been given those of the squash bug; but instead of developing the contamination that appeared so constantly in the squash bug eggs, they invariably remained sterile and showed no growth whatever, even when crushed immediately upon being placed in the bouillon.

Before undertaking the rearing work on *Murgantia* that was logically opened up by these negative results, it was decided to

determine just how long after hatching the infection really occurs under normal condition. A fairly complete series of the immature stages of this insect was available at the time, and starting with a half-grown nymph, the cæca of successively younger individuals were examined until a stage only a few hours old was reached without finding a single specimen free from the infection. The next step was to examine a nymph immediately after hatching and before there was any possibility of its having fed; and as the cæca of this specimen also proved to be infected, it became evident that the cæcal organism itself must be transmitted directly through the egg.

In order to establish this point beyond question, embryos of *Murgantia* in different stages of development were examined, and it was found that in an embryo taken as early as forty-eight hours before the time for hatching, the organism which had been found uniformly in all the post-embryonic stages of the insect also appeared in the embryo. The bacteria appear in that section of the gut which is due to develop into the cæcal apparatus of the adult insect and which, owing to its pink color, can readily be distinguished from the other embryonic divisions of the alimentary canal.

While the bacteria from the embryo of *Murgantia*, twenty-four to forty-eight hours before hatching, are comparatively very few in numbers, they nevertheless show the same characteristic, spiral forms that are met with in the adult, although those from the embryo are usually much shorter and decidedly more difficult to stain. A considerable number of embryos of this insect were examined in this way and, without exception, the cæcal region in all was infected with the same peculiar contorted organism.

These observations on *Murgantia* naturally suggested that the apparent contamination from the eggs of the squash bug might also have been due to the cæcal bacillus of this insect carrying through the egg in a similar manner; although the fact that no growth was secured from the eggs of *Murgantia*, in which the cæcal bacteria had been demonstrated microscopically, was rather unfavorable to this view. This fact also seemed to indicate that perhaps the cæcal organism was so closely adjusted to conditions in the body of the host insect that it would not develop readily on artificial media.

At this time no more eggs of the squash bug were available, and the cultures from them had all been discarded as of no significance; but the following season, when the matter was again investigated in the light of these new facts, it was found that the cultures that developed so regularly from the eggs of this insect were identical with those that were also secured from the cæca, and that instead of containing a number of contaminating organisms, the growth really consisted of a pure culture of the cæcal bacillus. The reason for believing, at first, that the cultures from the eggs represented more than one organism and the fact that no growth was secured from the eggs of *Murgantia* are discussed in connection with the regular culture work; and it is enough to say here that when ordinary care was taken in the disinfection of the eggs of *Anasa tristis*, the resulting growth was invariably a pure culture of the cæcal bacillus.

It was also established for a number of other species, including the lygæid, *Blissus leucopterus*, that the bacteria are present in the cæca of the nymph immediately after hatching, and consequently, at a time when all outside contamination could be excluded; but it was considered a piece of unnecessary routine to attempt to carry the examination back into the embryo of these forms as had been done in the case of *Anasa tristis* and *Murgantia histrionica*.

The exact manner in which the cæcal association developed in these insects can, of course, only be surmised. It is possible that the present normal bacteria were formerly pathogenic for the host, and that the cæca, as they now exist, originally developed from true pathological structures formed as a result of the invasion of the tissues by these organisms; or the bacteria may originally have been merely saprophytic forms, peculiar to this section of the alimentary canal, which gradually became adjusted to an existence in the cæca.

One factor that might be regarded as supporting the first view is the fact that the bacteria are, to a certain extent, intracellular; and it is easy to imagine how these organisms, originally attacking certain of the epithelial cells of the gut, might have stimulated the formation of pathological cæca, which, being incidentally of some use to the host, were preserved and even

greatly elaborated in structure. There is somewhat better reason for believing, however, that the cæca were originally independent organs which finally came to be occupied by the normal bacteria, and this subject will be taken up under the discussion of the cæca themselves.

That the infection is regularly transmitted through the egg from generation to generation is very evident from the above facts; but this does not necessarily mean that the organism is so closely bound up with the host as to exclude the possibility or even the importance of infection from without. One can hardly believe that reinfection by way of the mouth, whether an important factor in the association or not, is not constantly taking place, at least from the excrement of other individuals if not also from some undiscovered form of the organism that may exist normally as a free saprophyte.

As is well known, certain pathogenic blood parasites, as the spirochætes, are regularly transmitted through the egg from one generation to succeeding generations of the intermediate host, which is a tick in the case of *Spirochæta duttoni*. It has been established, in this particular case, not only that the mother that swallows the infected blood may transmit the parasites to her young, but that their descendants, although allowed to feed only on clean animals during their entire lifetime, may also pass this second-hand infection on to their young. Transmission may not stop even here, as it has been clearly established that it may continue in this way for at least three generations of ticks.

Just why this process should not go on indefinitely, when once the infection is acquired by the tick, is not clear; but evidently it does not, as only a comparatively small per cent. of the individuals of *Ornithodorus moubata* are able to transmit the disease to man.

In order for the association to continue, repeated infections with the blood-inhabiting forms of the spirochætes must apparently take place. It may be that conditions in the arthropod host are unfavorable in some way to the spirochætes, and that they gradually degenerate in passing through a number of generations of the host, so that for a continuance of the association a periodical reinfection with a more vigorous strain from some

outside source is required. While the occurrence of a similar relation in the Heteroptera is by no means certain, there are a number of facts pointed to both by direct observation and by analogy, that would seem to favor this view rather than the theory that the bacteria are confined strictly to their insect hosts and are transmitted indefinitely from generation to generation through the egg.

One reason for the first view is that in certain of these insects, as in *Murgantia* and in many other pentatomids, the bacteria really appear to be existing under marked difficulties of some sort, as indicated by the proportion of involution forms and the constancy with which they appear in the cæca of these insects, and also by the fact that the vast majority, at least, even in such forms as the squash bug, are apparently unable to develop any longer or artificial media. In the case of this particular insect, while growth was uniformly secured from the cæca where liquid media was used, it soon became evident, when plate cultures were made direct from these organs, that but a minute fraction of the bacteria actually present were able to develop on artificial media, the vast majority evidently being either dead or modified in such a way by their existence in the cæca that they were wholly unable to develop outside the host insect. Frequently when only a very short section of the cæca was removed for inoculation, especially where plate cultures were made, no growth whatever developed, although there were certainly thousands of bacterial cells introduced.

Another case that may throw some light of analogy on this question is that of the well-known symbiotic relation between certain green turbellarians and their associated algæ (Keeble, '07). Numerous attempts have been made to cultivate these algæ on artificial media, but uniformly without success. This was sometimes explained on the theory that these two organisms had been associated in this relation for so long that the alga had completely lost its ability to develop in the free state, and was now totally dependent on the animal, as is often the case in many of the more highly specialized parasites. This is certainly a very reasonable view but it has recently been discovered that the zoöchlorellæ, although exerting a profound influence on the

metabolism of the host, do not represent the typical organism any more than the *Leptus irritans* stage could be taken as representing the true life cycle of the particular species of *Trombidium* to which it belongs. The zoöchlorellæ, like *Leptus irritans*, really represent an abnormal departure from the regular development of the species. The form to which these symbiotic algæ belong is really a typical, free-living, flagellated organism, which is normally free during its entire, regular life cycle, the zoöchlorella stage representing merely those individuals which chance to be swallowed by the worm. The free-living stage of this organism will grow vigorously on artificial media and infection is found to take place readily from pure cultures, but in the case of the organism direct from the tissues of the host worm it has been found not only that they will not develop on artificial media, but that they have been so modified by their short existence in the body of the host, that they are not even able to set up the infection in clean worms although they may be swallowed in quantities.

In certain cœlenterates the relation of the alga to its host animal appears to be better established than in the planarians. In *Millepora* (Mangan, '09), at least, the algæ are found in the egg and appear to be transmitted through it for at least one generation; although there is a possibility that the inclusion of these organisms in the egg is a mere accident and that the chief source of infection is the free-living stage of the alga which is continually being swallowed by the animal.

The only other well-known bacterial infection in insects directly comparable with that in the Heteroptera is one whose relations have been worked out by Petri for the little olive fly, *Dacus oleæ*—a subject which will be discussed in detail in another place. In this insect the bacteria, which are also intestinal forms, are present in the larva as well as in the adult and, according to Petri, there is a complicated modification of the ovipositor which functions as a secondary reservoir for the intestinal bacillus. Petri at first very naturally reasoned, since these bacteria were present in larvæ that had hatched from eggs laid by sterile flies in sterile olives, that the organism must be transmitted through the egg; but in his later work he concludes that this is not the

case, and he finally decided that infection normally took place in the following manner: When the fly deposits its egg in the fruit of the olive, a quantity of the bacteria from the special structures in the base of the ovipositor are also introduced and infection only takes place when the young larva, upon hatching, swallows some of the surrounding bacteria. Petri also isolated a chromogenic organism from the soil of olive orchards and from various parts of the olive tree which he regards as identical with that from the intestine of *Dacus*, and he seems to think that the insect originally developed its infection from this free, saprophytic form.

The organism isolated so uniformly from the eggs and cæca of *Anasa tristis* clearly belongs in the large group of fluorescent bacteria that are so common in water and in soil generally. A number of strains of these saprophytic fluorescent organisms have been isolated and studied from the soil about squash vines and from the squashes themselves; and they clearly belong in the same group with the cæcal bacillus of *Anasa tristis*, although certainly none of them are identical with it.

Cultivation of the Cæcal Bacteria.

In undertaking a study of the physiological relation existing between the Heteroptera and their cæcal bacteria, it was very evident that the whole question hinged on the cultivation of these organisms, and that little or nothing could be expected from such a study until pure cultures were obtained. The fact that these were really normal bacteria and occurred in every individual possessing the cæca, in itself presented a very serious obstacle to the culture work, since this very fact apparently excluded all possibility of confirming, by direct infection experiments, any cultures that might be obtained from the cæca. An attempt was made, as has already been mentioned, to rear sterile individuals from the egg in aseptic cages, in order to secure material free from the cæcal bacteria for these infection experiments; but since the bacteria were found to pass normally through the egg, these rearing experiments were unsuccessful; and this apparently left no alternative but to select for the culture work only those insects in which the bacteria were so character-

istic in form that they could readily be recognized in mixed cultures.

Since all possibility of checking the cultures by infection experiments had to be definitely abandoned, there was very little inducement, at least at first, to attempt the cultivation of these bacteria from the Coreidæ or from such lygæids as *Blissus leucopterus*, in all of which they are short, uniform rods with nothing morphologically to distinguish them from dozens of other saprophytic bacteria that might occur as contamination in the cultures. While these insects appeared wholly unsuited for preliminary culture tests, many of the Pentatomidæ, as *Peribalus limbolarius*, *Brochymena quadripustulata*, and especially *Murgantia histrionica* were apparently ideal for this purpose, as the bacteria which they harbor, instead of being the small, typical bacillus form of the Coreidæ and of most of the Lygæidæ, are very characteristic in appearance, varying from the extremely long, straight, rod-like forms of *Peribalus* through the short, uniformly bent organisms of *Brochymena* to the remarkably large, characteristically contorted form uniformly occurring in *Murgantia*.

For this apparently good reason, the culture work was, at first, concentrated especially on *Murgantia histrionica* and a few other pentatomids; for it was very clear that if the cæcal bacteria from these insects would develop on artificial media at all, and still retain the characteristic form as they occur in the cæca, there would be no difficulty whatever in differentiating them from any possible contamination that might appear.

In the larger Heteroptera, as well as in those of moderate size, it was found that by careful dissection any division of the alimentary canal could be removed with practically no danger of outside contamination; the peculiar shape and structure of the abdomen in these insects being especially adapted to this operation.

For removing the cæca aseptically it was found, after a number of methods had been tested, that the following simple procedure was really the most satisfactory. The insect is first lightly chloroformed to prevent struggling, the wings are clipped off near the base and the whole body moistened with alcohol to remove the film of air and allow the penetration of the bichloride solution

which was usually used in the proportion of 1–500. The mercuric chloride solution is best applied with a bit of absorbent cotton held in a pair of old forceps. In this way the entire body of the insect can be thoroughly scrubbed with the disinfectant, so that any folds, such as those between the body segments, will certainly be moistened. After the bichloride solution has completely dried, which may be very well hastened by passing the insect back and forth before a Bunsen flame, the flat edges of the abdomen are clipped off, from near the posterior end up to the thorax, with a pair of fine scissors which have been previously flamed. The top of the abdomen immediately back of the thorax may be cut across with sterile scissors and the resulting flap formed of the entire dorsal wall of the abdomen may then be lifted back with a pair of flamed forceps, leaving the abdominal viscera exposed.

Usually in forms such as the larger Coreidæ and Pentatomidæ, the alimentary canal is considerably coiled in the posterior half of the abdominal cavity, and is covered above by a thin layer of fatty tissue which must be moved to one side before the cæca can be reached.

The cæca may be readily distinguished from the other divisions of the alimentary canal, as they are pure glistening white in the Coreidæ or tinted yellow or pink in many of the Pentatomidæ.

In making cultures from the cæca, the usual procedure was to open the abdomen with sterile instruments, in the manner just described, and after removing the dorsal fat body, to clip out a small section from near the middle of the cæcal system, which was then quickly removed with flamed forceps to a tube either of sterile salt solution or bouillon, the final cultures being made from this tube.

For reasons which have already been explained, the first serious attempt at cultivating the cæcal bacteria was made with *Murgantia histrionica;* and unfortunately the work was confined for a long time to this species and a few other pentatomids, in an attempt to discover some means by which their peculiarly characteristic bacteria could be made to develop on artificial media.

When the work was first undertaken with *Murgantia,* it was planned to remove a section of the cæca to a tube of sterile salt

solution or bouillon as just described, crush it, and from this make plate cultures in the usual manner, it being fully expected that a number of doubtful forms of bacteria would be isolated as contamination from the ordinary transient intestinal flora assumed to be present in the insects. It was a decided surprise, however, when not only the typical cæcal bacteria failed to appear in these plates, but no contaminating forms even developed. This was repeated with large series of the insects but the results were always the same, the cultures remaining sterile with a discouraging regularity. It was thought at first that perhaps the liberal amount of alcohol and bichloride solution used in disinfecting the outer surface of the insects might possibly have penetrated the tracheal system and that enough had been removed with the trachea adhering to the cæca to prevent all growth in the cultures, although this seemed hardly possible. Tests were made, however, in which only those parts of the insect which were actually to be cut were moistened with the disinfectant, the spiracles being avoided entirely, and the results were exactly the same, the cultures remaining uniformly sterile. It was later found also that without the use of any disinfecting solution the glands could be removed with no fear whatever of contamination from without, provided the scissors with which the cutting was to be done were used hot enough to sear as the body wall was opened; the cuts being made as rapidly as possible to avoid the danger of heating the tissues too deeply.

While the apparent incapacity of the cæcal bacteria from this insect to develop on ordinary media was somewhat discouraging, at least one very important fact was brought out by it. Since no growth at all developed from the cæca on ordinary media, it was very clear that, contrary to what had been expected, there would be no complication of contaminating organisms from the intestine which are so common in many other insects, since in *Murgantia* these forms seemed, for some very definite reason, to be wholly absent; and it followed that, if by any means conditions could be made suitable for the development of the cæcal bacteria of this insect on artificial media, the difficulty of proving that the organism in culture was really the one sought and not some accidental contaminating form would be wholly avoided.

With this as an encouragement, an excessive amount of time and effort was wasted in an attempt so to modify culture conditions that the normal cæcal organism of this insect could be induced to grow on artificial media. Since, in reaction, the cæca appear faintly alkaline, the different media used were usually made neutral or slightly alkaline; and as the normal food of the insect is cabbage, this was largely used in the different media tested; but as all the results were uniformly negative, no minute discussion will be given here of the different modifications that were tried. Anaerobic cultures were also made with negative results, although at the time, this test seemed almost superfluous in view of the abundant tracheation of the cæca.

After the failure of these direct culture experiments, it was reasoned that upon the death of the insect the bacteria probably became gradually adapted to a saprophytic mode of life, and that by taking advantage of this they might still be forced to grow on artificial media.

In testing this hypothesis, several series of from forty to fifty insects each were used. The insects were killed either with chloroform or by grasping the head for an instant with a pair of very hot forceps, the last method being the one most generally used and proving a very convenient way of killing the insect without breaking the body wall. The dead insects were then thoroughly sterilized by washing in a mercuric chloride solution after the removal of the wings, and after drying they were placed in small, tightly stoppered, sterile vials, to prevent drying of the internal structures, and kept for three or four days in a cold box at about 20° C. At the end of this time the alimentary canal was usually intact and showed no invasion by foreign bacteria, while the cæcal bacteria themselves showed no perceptible changes either in numbers or in the invasion of other regions. Bouillon tubes inoculated from the cæca of these dead bugs usually remained sterile, but growth appeared in two or three per cent. of the tubes, and it was thought at first that this growth might represent strains of the cæcal organism which had been modified by their stay in the dead bugs so as to develop on artificial media. When these cultures were examined under the microscope, however, they showed, not the long, irregularly bent

organism from the cæca of *Murgantia*, but what appeared to be a pure culture of a very short, actively motile, fluorescent bacillus, which grew vigorously on ordinary media. Although this organism was obtained in culture from ten or more of the dead insects and appeared to be the only one that developed, it was so different from anything that had been expected from the cæca that it was regarded as one of the common fluorescent water bacteria that had probably been present in the anterior part of the alimentary canal and which, after the death of the insect, had invaded the cæca. The culture work on *Murgantia* was discontinued here, as it apparently promised nothing to warrant any further work.

In the meantime repeated attempts were also made to cultivate the cæcal bacteria from a number of other pentatomids, including chiefly *Peribalus limbolarius*, *Cœnus delius*, *Brochymena quadripustulata*, *Euschistus variolarius* and *Mormidea lugens*, but the results, as with *Murgantia*, were uniformly negative, neither the cæcal organism nor any contamination appearing in the cultures from any of these species, except in exceptional cases where the technique was clearly at fault.

Common forms such as the squash bug and the chinch bug had been purposely avoided in this work owing to the obvious impossibility of distinguishing between ordinary contaminating organisms and the cæcal bacteria typical for these insects; but since the negative results from the work with *Murgantia* and the other pentatomids showed clearly that the cæca, in these insects at least, were wholly free from foreign bacteria, the possibility of using such insects as *Anasa* in culture work did not seem so hopeless as at first, especially if it was found that this statement applied also to them.

As a last resort it was decided to attempt the cultivation of the cæcal bacteria from *Anasa tristis*, this species being selected because it is fairly large and is usually abundant and readily obtainable in winter as well as in summer.

In the preliminary work on this species, pieces of the cæca were removed and dropped at once into tubes of squash juice bouillon, the composition of which was the same as that of ordinary beef juice bouillon with the addition of a decoction from 150 grams of squash stems and leaves per liter.

In the squash bug the cæcal bacillus is a very short, uniform rod, averaging 0.9 micron long by 0.7 micron wide. As they occur in the cæca these bacteria do not show the slightest indication of motility; and they are usually arranged in pairs, or they may, exceptionally, form short chains of from three to four or more individuals.

In undertaking the culture work with this insect I had very little hope of growing the cæcal bacteria successfully, and really did not expect more than a repetition of the negative results secured with the Pentatomidæ, but I nevertheless thought that even the relation shown there would be worth establishing in other Heteroptera.

The first culture experiments with *Anasa tristis* were undertaken with seven adults that had been kept in a warm room for nearly a month. Of the tubes inoculated from the cæca of these seven insects, every one developed growth; and in each case this appeared to be a pure culture of an organism morphologically very similar to the bacteria in the cæca; but as these insects had been feeding on partly decomposed squash a short time before the dissections were made, it was realized that the growth might very easily have been due to some foreign organism swallowed by the insects while feeding. Nevertheless, the fact that the growth in all the tubes was apparently the same, was very encouraging in view of the uniform failure to secure any growth whatever from the Pentatomidæ.

To ascertain whether or not this growth was merely a contamination from the squash, or whether the cæcal organism could still develop saprophytically, as the cultures suggested, a series of fifty specimens of *Anasa* were taken, fed on perfectly fresh squash for two weeks and then kept for a full week without food, in order to give the cæcal bacteria sufficient time to destroy any contaminating organisms that might have been swallowed while feeding.

Cultures were made from this series and, to my surprise, growth developed in all fifty of the tubes and appeared in each case to be a pure culture of a short, motile organism identical in size and form with the bacteria in the cæca of this insect, and so far as could be determined these were the same as the

organism that had appeared in the cultures from the first seven insects tested.

This series of experiments appeared to show very conclusively that we had at last succeeded in growing the cæcal bacteria in pure culture, provided of course that foreign bacteria were excluded as completely from the alimentary canal of *Anasa* as they had been shown to be in the Pentatomidæ. Subcultures were not started from these tubes for seven or eight days and upon examining them again at the end of this time, a number were found to contain organisms so different from the cæcal bacteria that there was apparently no escape from the conclusion that the cultures had become in some way contaminated. When examined upon the first appearance of growth, nothing was found but the short uniform bacillus, which was as much like the bacteria direct from the cæca as could be imagined; but at a later examination two apparently distinct contaminating forms were discovered. One of these apparent invaders was a fairly uniform rod-shaped bacillus 4–8 microns long by 1 micron wide, so much larger and longer than the typical cæcal organism that the two could be distinguished at a glance. The other was a perfectly spherical form, 0.5–0.7 of a micron in diameter, which occurred commonly in extremely long chains of a hundred or more individuals.

Contamination from some source had apparently taken place in a number of tubes, and it was therefore decided to discard the whole series rather than attempt to make a detailed study of the different forms that might be isolated from the cultures in an attempt to connect some one of these with the species from the glands—an undertaking evidently little short of hopeless. Another cause of uncertainty here was the fact that the cæcal bacteria from the Pentatomidæ would not develop on ordinary media; and as there was no reason whatever for assuming that the forms from the Coreidæ would behave any differently, there was clearly a possibility that the growth that had appeared in all the tubes was really contamination and that the true cæcal bacillus was not represented here at all.

If the property of totally excluding all other bacteria from the alimentary canal did not hold for the cæcal bacteria of the

Coreidæ, as it very clearly did for those of the Pentatomidæ, then there was certainly very little encouragement to continue culture work with these insects, for although strains of bacteria might be isolated that would agree perfectly with the cæcal bacteria in their morphological characters, this would establish very little, as there is nothing about the bacteria from the Coreidæ to distinguish them in this way from many common saprophytic forms that might readily gain access to the intestine of these insects.

As the first two sets of culture tests were made from bugs that had been kept in a warm room and had been feeding continually up to a week of the time before the dissections were made, it seemed possible that the antagonism of the coreid bacteria to invading forms was not so absolute as in the Pentatomidæ, and that, if hibernating insects were used which had had no opportunity to feed for a considerable period, the normal bacteria might in this time have succeeded in effectually killing off the foreign species. It was accordingly decided to attempt the cultivation of the squash bug bacillus once more, hibernating insects being used this time in the hope that as a result of long fasting their normal bacteria might have eliminated the transient forms.

For these tests, the insects were taken after the middle of December from their winter quarters. They could not have fed for at least a month; and they were dissected at once. Thirty-five specimens of *Anasa tristis* were collected for this work and every precaution was taken in the dissections and inoculations to guard against contamination from without. The insects were sterilized before dissection with a thoroughness which would not have been permissible with *Murgantia;* but, notwithstanding this, cultures were obtained from these insects as regularly as they had failed where *Murgantia* was used, all thirty-five of the tubes inoculated developing an abundant growth.

These cultures were incubated at room temperature and were watched closely for the appearance of any contamination. Growth was found to appear rather tardily, developing in from two to four days, and at first, as in the two previous series of experiments, consisting of an apparently pure culture of a short, motile organism which could not be distinguished morphologically from the cæcal bacteria as taken direct from the insect. In

a few days after the first appearance of growth, however, forms were observed in many of the tubes which were clearly not of this type. In about a week the two abnormal forms which had appeared in the preceding experiment were observed in a number of these tubes; and, in addition to these, a third type was also discovered which appeared in several of the tubes as a very large, oval, yeast-like organism often over 4 microns long by 2–3 microns wide.

As all contamination from without had been excluded beyond question, all of these unusual forms must certainly have come from the cæca; and as nothing resembling any of them had ever been observed in these organs by direct examination, their appearance in the cultures was very difficult to explain.

Upon close examination it was seen that these contaminating organisms were not strictly constant in form, although it did not seem possible at first that these strikingly different organisms could be involution forms of the bacillus that first appeared in cultures from the cæca. The yeast-like bodies were usually free, with one or more small typical buds at the ends; but occasionally one or more of these large bodies could be seen in the long chains of small coccus-like forms; and the large bacillus was not always a typical rod, but very often tapered at the ends very decidedly, and even merged into bodies that resembled the yeast-like forms. Since it seemed possible that some of the contaminating organisms were merely involution forms, and not independent organisms, as had been assumed at first, it seemed worth while to make a thorough study of the different forms occurring in the cultures to determine how much normal contamination there really was in the alimentary canal of these insects. Plate cultures were accordingly made from some of the tubes that appeared most highly contaminated, and these were searched very carefully for different kinds of colonies; but so far as could be determined by direct examination, the colonies were invariably all alike.

Cultures were made from large numbers of these colonies in an attempt to isolate the different contaminating organisms that had appeared in the original bouillon tubes. All the subcultures from the plates invariably developed into the short form resembling the cæcal bacillus; but when subcultures were

made from these into bouillon, all the abnormal forms regularly appeared, as they had in the cultures direct from the cæca. Since each colony in the plates contained only descendants of a single bacterial cell, it became very evident that the apparent contamination from the cæca of the squash bug was nothing more than an extreme case of the production of involution forms, and that but a single organism had ever really developed in cultures from the cæca of these insects. This established conclusively the fact that we had but one organism to consider in the culture from the cæca of *Anasa tristis* although it did not show beyond question that this one organism was really the true cæcal bacillus. In view of the results from the culture experiments with *Murgantia*, however, it certainly seemed probable that this was the cæcal bacillus.

One fact which made this view rather uncertain for a time was that in 1895 a generally distributed disease of the squash bug was discovered by Duggar in the neighborhood of Urbana. This disease appeared to be fairly common, and was described as affecting principally the fat body and the perivisceral tissues generally. There was a distinct possibility that a chronic form of this disease might be present among the apparently healthy bugs that had been used in the culture tests just described, and that the growth in these cultures was really due to this organism from the fat body contaminating the cæca as they were removed from the insect for inoculation.

No more insects were available for testing this theory, but the following season several large series of culture tests were carried out with this point in view, two tubes being inoculated from each insect in the following manner: Upon opening the abdomen a large lobe of the fat body adjacent to the cæca was removed, before the alimentary canal was broken, and placed in one of two tubes of bouillon. A section of the cæcal system was then removed and put into the check tube. Without discussion of detail, it is enough to say that as a result of several hundred such tests growth was obtained from the cæca in every case, while the tubes inoculated from the fat body invariably remained sterile.

In order to ascertain the distribution of the cæcal bacteria

throughout the alimentary canal, cultures were made from different sections of the midgut, including the first stomach, third stomach and the cæcal region. From a large number of such tests it resulted that while growth invariably appeared in the tubes from the cæca, and usually also from the third stomach, only from ten to twenty per cent. of the inoculations from the first stomach showed any growth, all the others remaining sterile.

Cultures were repeatedly made from the eggs of *Anasa tristis;* and upon comparison, it was found that the organism from this source was certainly the same as that isolated from the cæca, and it was also determined that this was the only organism that ever appeared in cultures from the eggs of this insect.

It was expected, in the beginning, that the bacteria from the cæca of the Heteroptera, if they developed at all on artificial media, would prove to be a number of very different forms, perhaps occurring only in their respective insect hosts, and when it was found that the organism isolated from the cæca of *Anasa* belonged to so common a group as the non-liquefying fluorescent bacteria, it was feared that, after all, the form isolated from this insect might have been merely the result of a contamination of the alimentary canal by some of the ever-present species of this group; although the fact that this same organism was isolated regularly from the egg seemed very conclusive.

In order to show beyond question whether or not the bacteria so regularly isolated from the squash bug were really the same as those normally present in the cæca, it was planned to check the cultures which had been obtained from the insects against the bacteria direct from the cæca by means of the agglutination test. Young rats were chosen as best suited for this work, as they are much more hardy than young guinea pigs of the same weight. The animals selected weighed only from fifty to seventy-five grams, as it was feared that sufficient material for the immunization might not be available in case large animals, such as full-grown guinea pigs, were used. It was planned at first to immunize the animals to cultures of the bacillus isolated from the cæca, and to test such sera against emulsions of the bacteria direct from these organs, but owing to the fact that bacteria

freshly isolated from tissues do not usually react so readily to the agglutination test, the process was finally reversed and the animals were immunized with material direct from the cæca, the crushed cæca really representing a greater bulk of bacterial cells than of insect tissue.

For this purpose the cæca were removed from hibernating insects and crushed very thoroughly by rolling between sterile slides. The bacteria thus liberated were then washed off the slides and collected in sterile vials which were at once immersed in a water-bath and kept at 54° C. for thirty minutes to kill the organisms, it having been found that this temperature was sufficient for the purpose.

Material prepared in this way was injected intraperitoneally, each animal receiving five graded doses at intervals of a week or ten days, the doses varying from the cæca substance of ten bugs for the first injection to that from thirty insects for the last.

The immunized animals were killed about ten days after the last injection, the blood was drawn aseptically from the heart, and the serum stored in capillaries having a capacity of two or three drops each. Since the normal serum of many animals is known to agglutinate certain strains of bacteria in low dilution, I did not know what to expect in a case like this, and to guard against any possible error, a normal rat was bled whenever an immunized animal was killed and the sera from the two were checked against one another in every test made.

A large series of cultures from the cæca of *Anasa tristis* were tested out against such immune sera, and from the very first of these tests it was evident that the organism in culture was undoubtedly the identical form in the cæca, for they were agglutinated readily by the immune serum of the cæcal bacteria in dilutions as high as 1–500. No dilutions higher than this were tried, but from the readiness with which clumping was produced in this concentration, we may infer that the reactions would certainly have been reliable in dilutions twice as great.

In this work the macroscopic method was relied upon almost entirely, the dilutions being made in small, 6 or 7 mm., test tubes. It was found that while serum from the immunized rat would regularly produce complete clumping in high dilutions in

three or four hours, when incubated at 35° C., the normal serum in these same dilutions gave no trace of the reaction. It showed no more flocculation than regularly took place in the untreated checks, although there does often appear to be a slight reaction in dilutions as high as 1-20 where normal rat serum is used.

From a careful study of the bacteria as they occur in the cæca of the different Heteroptera, supplemented by the results of this culture work, it seems highly probable that many, and perhaps all, of the different and highly characteristic forms observed in the cæca of so many of these insects are really abnormal involution forms of an organism presumably something like the cæcal bacteria of *Anasa tristis.* This cannot be certainly determined, however, until at least a few of these peculiarly shaped organisms have been cultivated beyond question. It will be remembered that during the culture work with the cæcal bacteria of *Murgantia histrionica*, growth was secured from a small per cent. of the insects that had been dead for some time. The organism isolated in this case being a short, motile form, very similar in the morphological and cultural characters recorded to the bacteria from *Anasa tristis.* As there appeared at that time, however, no way of determining positively whether these really represented the true cæcal organism of the cabbage bug or were merely accidental invaders which had appeared after the death of the insect, the cultures were all discarded, as has already been mentioned, after having been studied in only a very superficial way.

Evidently the only method available in a case such as that described for *Murgantia* is the agglutination test as applied to the cultures from *Anasa tristis*—immunization with the bacteria of the particular insect studied, direct from the cæca, and testing the resulting serum against any doubtful cultures obtained from these organs.

I have had no opportunity as yet to repeat this work with *Murgantia*, but expect eventually to make a careful comparative study of the cæcal organisms from a number of host species, concentrating especially on such forms as *Murgantia*, *Peribalus limbolarius*, *Peliopelta abbreviata*, or others in which the normal organism is equally characteristic; the agglutination test, of course, being used as a basis for the work.

It was firmly expected, when the present investigation was undertaken, that the characteristic and remarkably varied types of cæcal organisms observed in the different Heteroptera would be found to represent a large number of different species and perhaps genera, and that a very careful study and description of each type, at least as they occur in the cæca, would be necessary. In view of the results which have developed from the culture work, however, it seems probable, almost certain in fact, that all of these strikingly different forms really belong to a single clearly defined group of bacteria, and that the differences in structure that are so constant for the given host species are due to some specific physiological peculiarity of the insect which exerts a very definite influence in determining the morphology of the bacterial cell.

While all of the different strains isolated from the cæca and eggs of *Anasa tristis* belong to the group already mentioned, they are by no means all identical in their behavior on artificial media. The most striking of the differences observed was in the vigor with which they developed in culture, some strains showing a remarkably strong growth from the very beginning, others making a comparatively very weak growth even after repeated transfers, while occasionally a strain would be found which, growing very feebly at first, gradually weakened for some unknown reason and finally refused to grow altogether after three or four transfers.

Another thing that I have not yet been able to do is to make a detailed comparison of the cultural characters of the different strains isolated from the squash bug, but from the observations already made there is undoubtedly considerable variation in the minor cultural characters in many of these.

Quite recently in working with other coreids than *Anasa tristis*, cultures have been secured regularly from the cæca of *Alydus quinquespinosus*, *Aldyus conspersus*, and *Metapodius terminalis*, as well as from the eggs of the first species of *Alydus*, although cultures attempted from the eggs of a single female of *Archimerus indecorus* were uniformly negative.

While the bacteria isolated from these three coreids show certain slight differences in culture from the typical strains from

the squash bug, they certainly all belong to the same group and the differences noted were no greater than those observed in different strains from the squash bug itself.

It was thought at one time the bacteria isolated from the cæca of these insects might readily be identified as some already described species known to occur free in nature, but the classification of this group was found to be in such a chaotic state that this idea was abandoned; and for the same very good reason it was thought that little would be gained by an attempt to describe them as new.

Some idea of the striking morphological differences in the bacteria from different host species can be gained from the following brief notes on a few of the more characteristic types as they occur in the cæca. No attempt will be made in this article to mention all of the many different morphological types of bacteria that have been observed and studied in the different host species dissected, the present object being merely to give examples of a few of the extreme cases that are met with. For this purpose the cæcal organisms from *Anasa tristis*, *Blissus leucopterus*, *Euschistus servus*, *Peribalus limbolarius*, and *Murgantia histrionica* should serve very well, as in this series we get the two extreme types in *Anasa* and *Murgantia*, the others representing intermediate forms.

In the first two host species the normal bacteria are much alike in appearance, both being very short rods which average something like one micron by 0.7, those from *Anasa tristis*, however, being usually slightly smaller and rather more slender than those from the chinch bug (Plate VIII., Figs. 21 and 23). In both of these insects the bacteria are quite uniform in appearance, varying no more in size and form from the typical cells than might well be expected. They occur very regularly in short chains of two, occasionally of four and very rarely of six or more cells, and do not show the slightest indication of motility as they come from the cæca. They take the common stains readily and on the whole have the appearance of ordinary bacteria with nothing especially striking to characterize them.

From *Euschistus servus* (Plate VIII., Fig. 24) the bacteria are decidedly longer than in the insects just mentioned, although

they still have the appearance of ordinary bacteria. As in the two preceding forms they occur typically in pairs and show no motility. The individuals of these pairs average about 4 by 0.9 microns, while some may be considerably longer. Individual rods, which are often slightly bent, may be as long as 8 microns while short rods no longer than 1.5 microns are frequently present, although these different forms all grade insensibly into one another.

In *Peribalus limbolarius* (Plate VIII., Fig. 25) the bacteria are very much longer than in *Euschistus servus*, and while they still tend to appear in pairs this feature is not so marked as in the three cases mentioned above. The individual cells are remarkably long, varying from 5 to 50 microns long by about 1.2 microns in diameter. The shorter rods often show a characteristic bending, the curve being very gradual and extending the full length of the cell. This tendency is greatly exaggerated in the longer elements, which are frequently bent several times, but even here the curves are gradual and symmetrical, there being no sharp twisting and distortion of the rods as in *Murgantia histrionica*, and even in the longest, the diameter remains very constant throughout. The extremely long elements are more common in some individuals than in others, frequently making up a large part of the cæcal contents in such insects.

When these organisms were first encountered I could hardly believe that the extremely long, curved, thread-like structures really represented single cells and not chains of closely packed units, although no definite divisions could be made out either in fresh or in stained preparations. When stained heavily and then decolorized to a certain point these threads break up into irregular bead-like granules which are scattered rather regularly throughout. These granules clearly do not represent separate units, however, as the same bodies likewise appear in the short rods under the same manipulation, and these unquestionably represent single cells as do also the longer thread-like elements.

The cæcal organism of *Murgantia histrionica* (Plate VIII., Fig. 26) is of such bizarre form that there is really little about it to suggest its bacterial nature, and this might very well be questioned, in the absence of anything definite in the way of culture

tests, if it were not that there is a more or less complete transition from it to the typical bacterial forms of such insects as *Anasa tristis* and *Euschistus servus.* The transition forms are found in such pentatomids as *Brochymena quadripustulata, Proxys punctulatus* and *Peribalus limbolarius.*

There is little to characterize the cæcal organism of *Murgantia* except its irregularly twisted and remarkably contorted form, which is retained not only in the cæca, but also in the embryo during its passage through the egg. The irregular form of this organism as it occurs in the cæca, which is much more suggestive of spirochætes than of bacteria, does not lend itself well to description and the reader will perhaps get a better idea of this by glancing at the figure referred to above.

These organisms vary from small, contorted individuals, 2 or 3 microns long to huge, irregularly bent bodies, not infrequently measuring 100 microns or even more in length by 1.5 to 3 microns in diameter. The most common form is 10 to 15 microns long although examples 25 to 30 microns long are common. The diameter of a single individual may vary considerably at different points and frequently such elements with a very decided bulb-like enlargement at one end, or even in the middle, occur. Others which are decidedly swollen throughout and which stain with great difficulty are fairly common, while often an end of one of the long individuals will take the stain readily, the remainder staining weakly or not at all. On the whole these organisms from *Murgantia* strongly suggest degenerating, involution forms, and this is probably their true nature.

Function and Phylogenetic Significance of the Cæca of the Heteroptera.

The midintestine of the Heteroptera is typically divided into four rather clearly defined regions which, for convenience, may be termed the first, second, third and fourth stomachs. All four can usually be made out, although in some of the more highly specialized groups certain of these divisions, especially the two posterior ones, may be very greatly reduced.

At its anterior end the midgut is uniformly dilated, forming a capacious, thin-walled, bag-like structure, capable of consider-

able distension. In the adult this anterior division is almost always empty, although in the nymph it is often filled, and even considerably distended, with a greenish or brownish granular mass, and, as might be expected, it is variable in size and shape in different individuals of the same species. At its posterior end this first stomach narrows suddenly and passes into the comparatively slender, tubular second stomach which usually is of uniform diameter throughout, and which empties into a second, oval or rounded, dilated portion. This third stomach, in turn, passes abruptly into the fourth and last division, which is the one that concerns us most, as it is on this section of the gut that the cæcal appendages with their normal bacteria appear.

The first stomach is almost always distinct, while the second and third may occasionally grade into each other, in the more specialized forms, in such a way that the exact line separating the two cannot be clearly made out. This fusion is brought about either by the excessive enlargement of the tubular second stomach or by the contraction of the third, or sometimes by both, and is met with only in those groups in which the cæcal appendages are wholly wanting. In those groups in which the cæcal appendages are regularly absent, the fourth stomach is uniformly reduced to an extremely short tubular portion, or it may occasionally be absent altogether, in which case the Malpighian tubes are inserted immediately below the third stomach.

In certain of the strictly predaceous groups, as in the Reduviidæ, Phymatidæ, and Acanthiidæ, the midgut is very greatly reduced in complexity, there being no trace of the fourth and very little to indicate the second and third divisions, and even the first stomach may sometimes merge into the remainder of the midgut to form an irregular tube of large caliber extending from the œsophagus to the rectum, but without any clear-cut divisions. Even in cases such as this, however, there is usually a slight enlargement at the posterior end, just in front of the point of insertion of the Malpighian tubes; and this may represent the third stomach as it occurs in typical forms.

The fourth division of the midgut is never well developed except in those families of the Cimicoidea which are provided with cæca, and so far as is known these include only the Pentatomidæ,

Thyrecoridæ, Pyrrhocoridæ, Lygæidæ, and the Coreidæ. In these families, whenever the cæca are present, the fourth stomach occupies a prominent place in the midgut in the form of a long, slender tube, and it is on the posterior end of this tube that the pouch-like cæca are borne. In these forms the tubular part of the fourth stomach is regularly somewhat dilated immediately below the third stomach, and this tendency is occasionally exaggerated to such an extent that in certain species, as in *Blissus leucopterus*, a bulb-like structure may be formed at the anterior end of the tube nearly as large as the third stomach itself, but this dilation is not constant in size and does not represent one of the typical divisions of the midintestine.

The ileum in the Cimicoideæ presents a characteristic modification which may well be mentioned here, for instead of continuing as a simple tube from the point of insertion of the Malpighian vessels to the rectum, it forms a thin-walled, bladder-like reservoir, of various shapes, into which the four Malpighian tubes empty, each tube usually being inserted singly, about half way down on the side of this bladder-like ileum. The ileum opens by way of a narrow, valve-like constriction into the capacious, thin-walled, muscular rectum, which may or may not be provided with a large anteriorly projecting cul-de-sac, depending on whether the ileum opens directly into its anterior end or further down on its side as it does in some of the Coreidæ. The walls of the rectum are very elastic and its size varies greatly. When not gorged it may be no larger than the third stomach and still be smoothly rounded, while in the same species it is occasionally found so greatly distended that it occupies most of the abdominal cavity, although this condition is decidedly exceptional.

As has already been stated, the cæcal appendages of the Heteroptera are apparently confined strictly to certain families of the Cimicoideæ; and while these organs at first appear to form a number of widely different types, they are found upon closer examination to fall regularly into two clearly defined groups ranging from very simple to highly complex forms. In one of these groups in which the Pyrrhocoridæ, Thyrecoridæ, Lygæidæ, and Coreidæ may be included, the cæca are arranged in two rows

extending along opposite sides of the tubular fourth stomach, in varying degrees of complexity, from an extremely simple type found in the Pyrrhocorinæ (Plate IV., Fig. 10) (in which the cæcal equipment consists merely of a double row of a half dozen or more comparatively minute outpocketings at the extreme posterior end of the tube, and this only in the female), to the highly complex arrangement met with in the Largiinæ (Plate VI., Fig. 16) and in many of the Coreidæ and Lygæidæ (Plate I., Fig. 1) (where the cæca may take the form of two rows of short closely packed units often numbering into the hundreds), or they may be arranged in definite groups of extremely long finger-like tubes, on opposite sides of the intestine, much fewer in number than in the forms just mentioned, but compensating largely for the loss in numbers by a very marked gain in the diameter and length of the individual cæca. In all of these highly modified forms the typical arrangement is maintained. In the other group, including the Pentatomidæ, with the exception of the Asopinæ in which the cæca are absent, there are four rows of short, uniform, closely set, sac-like structures ranged along the tubular portion of the fourth stomach; and this arrangement is adhered to with remarkable uniformity in all the typical Pentatomidæ so far examined.

Before discussing the various forms of cæca in detail, it might be well to consider the probable origin and course of development of these complex organs in the different divisions of the Cimicoidea.

Recognizing the Pentatomidæ as the more primitive Heteroptera then, according to the view expressed by some authorities, the Asopinæ might be taken as representing the stock from which all the other Heteroptera developed. We find in examples of this subfamily, such as *Podisus maculiventris* (Plate II., Fig. 5), that the cæca are wholly absent, and that the fourth stomach consists merely of a short neck connecting the third stomach with the ileum. If we consider the Asopinæ as representing a type which existed previous to the first appearance of cæca in the Heteroptera, then the Thyrecoridæ should logically come next in the series, as they form a perfect connecting link, so far as the cæcal structures are concerned, between the typical Pentatomidæ

with their four rows of cæca and the Lygæidæ, Coreidæ and Pyrrhocoridæ with their two rows. In this family, as represented by *Thyrecoris unicolor* (Plate I., Fig. 3), the cæca might well be regarded as showing a very primitive condition, since they appear in a double row of very blunt evaginations from the wall of the gut which shows no indication of the distinct, narrow ducts so common in most of these insects, and which might readily be imagined as in process of formation directly from folds or wrinkles in the intestinal wall. No direct transition has as yet been seen between the double row of cæca of *Thyrecoris* and the quadruple arrangement in the typical Pentatomidæ; but it can hardly be doubted that in some of the Thyrecoridæ, or perhaps in the Cydninæ, forms will be discovered in which the doubling of the rows of cæca is actually taking place. The specimens of Thyrecoris examined, and from which the drawing was made, were males; and as the cæca uniformly show a more complete development in the female than in the male, I should not be surprised to find at least indications of a third and fourth row when this sex is examined, although in the male the intestine does not show the slightest indication of this, even in sections.

This arrangement apparently does very well in the Pentatomidæ; but when the other families in which cæca occur are considered, a number of almost insurmountable difficulties are encountered.

It is easy to imagine how the complex types of cæca in the Coreidæ, Lygæidæ, and Pyrrhocoridæ could have originated from a common form like that of *Thyrecoris*, but the fact that in each of these families a number of species occur which are obviously not primitive, but in which the cæca are wholly wanting, is decidedly confusing when a progressive development of the cæca is considered.

It is probable that these organs, instead of representing a continuous line of development in the existing Heteroptera from simple to complex types, are really primitive, ancestral organs, as has been suggested by Mr. C. A. Hart, of the State Laboratory of Natural History, which are actually in process of elimination as the group advances in specialization; and that the confusing cases met with in the three families just mentioned should really

be regarded as more highly specialized forms in which the cæca have entirely disappeared. This view seems to agree in essentials with the best grouping of the Heteroptera, and Mr. Hart, who has kindly compared the evidence presented by the structures with the principal systems of classifications that have been proposed for these insects, finds that the relationships indicated by these structures agree remarkably well with the grouping of the Heteroptera as proposed by the great European hemipterist, Stål, although suggesting relationships decidedly at variance with those that have been assumed by certain American workers.

The view that the complex types of cæca should be considered the more primitive is also strongly supported by embryological evidence.

Upon dissecting the embryo of the pentatomid, *Murgantia histrionica*, a day or more before time for hatching and shortly after the midgut had formed, the cæcal region was found to be represented by a comparatively long, pink section of the intestine, upon which the four rows of minute cæca were appearing, each cæcum originating as an independent evagination of the wall of the embryonic intestine.

A similar study of the embryonic gut of such Lygæidæ as *Blissus leucopterus* would be very desirable, in order to see if the typical grouping of the cæca of the Blissinæ is not merely a specialization from forms such as those in the Pachygronthinæ. An examination of the embryonic cæca of the Thyrecoridæ as well as of the Cidnidæ, would also be worth while, as it is here that we should expect the actual transition to take place from the four-rowed form of the typical Pentatomidæ to the forms with but two rows, if the cæca in the Heteroptera are really being reduced in complexity.

If this view is correct, and it can hardly be questioned, then the Asopinæ could not be considered the more primitive type of the Heteroptera, as has been suggested by Kirkaldy, but would have to be considered a much more specialized group than the Pentatominæ, in which the cæca reach their greatest development in such forms as *Brochymena quadripustula*, where there may be as many as 1,400 cæca in the entire system.

Just why the cæca should drop out so suddenly in the Asopinæ

is not clear, unless this is due to a specialization of the alimentary canal correlated with the strictly predaceous habits of these insects. In this connection, it is of interest to note that the cæca are invariably absent in all the strictly predaceous Heteroptera.

The Scutellerinæ, judging from their cæcal equipment, are evidently close to the primitive Pentatominæ. Of the Cydninæ but a single male of *Amnestes pusillus* has been examined; and as the cæca were apparently absent in this specimen, this subfamily may be further removed than even the Thyrecoridæ, although no generalization can be made from this one dissection.

In regard to the function of the cæca of these insects, it can only be said that no digestive function has been discovered, and that the food mass in process of digestion apparently never gets beyond the third stomach.

This, together with the fact that these organs are located at the extreme lower limit of the digestive portion of the gut and are apparently in process of elimination, would seem to show that they possess no important digestive function, although the great development of these structures in forms like *Brochymena* would certainly suggest this.

It seems not improbable that the present function of the cæca of the Heteroptera is merely to provide a safe place for the multiplication of the normal bacteria of these insects.

It was originally planned to include in the present paper a discussion of the histology of the different divisions of the alimentary canal, as well as a comparative treatment of the cæca from the different insects studied, having in mind particularly the bearing of these structures on the relationships and classification of the different groups of Heteroptera; but owing to lack of available space this aspect of the work will have to be omitted for the present and reserved for publication at another time. A glance, however, at the accompanying illustrations of typical cæca selected from a few of the Heteroptera dissected, will show very well the range of development of these organs in this group, and also the possibilities that open up for a very thorough and complete survey of the cæca in these insects.

Some idea of the ground already covered in this phase of the work can be had from the following list of species, representa-

tives of all of which have been dissected and studied; each species usually representing a large number of individual dissections.

It will be highly desirable to dissect a much wider range of species than is included in this list before making any final generalizations, and all alcoholic or preferably living material that any reader may be able to contribute to supplement this list, especially in the Lygæidæ or Coreidæ, will be very welcome indeed.

Many of the species already dissected were obtained through the kindness of Mr. C. A. Hart, of the State Laboratory of Natural History, to whom I am greatly indebted. I wish also to thank Mr. L. M. Smith and Mr. W. P. Flint, both of the Illinois state entomologist's office, for much valuable assistance in securing material for the work.

The following are the species that have been dissected so far in connection with this problem:

Pentatomidæ.

Brochymena quadripustulata Fabr.
Euschistus fissilis Uhl.
Euschistus servus Say.
Euschistus variolarius Pal. Beauv.
Murgantia histrionica Hahn.
Nezara hilaris Say.
Thyanta custator Fabr.
Thyanta preditor Fabr.
Peribalus limbolarius Stål.
Hymenarcys æqualis A. & S.
Œbalus pugnax Fabr.
Cosmopepla carnifex Fabr.
Neottiglossa undata Say.
Mormidea lugens Fabr.
Cœnus delius Say.
Proxys punctulatus Pal. Beauv.
Podops cinctipes Say.
Podisus maculiventris Say.
Perilloides circumcinctus Stål.

Scutelleridæ.

Homoemus proteus Stål.

Cydnidæ.

Amnestus puscillus Uhl.

Thyrecoridæ.

Thyrecoris unicolor Pal. Beauv.
Thyrecoris pulicaria Germ.

Coreidæ.

Archimerus indecorus Walk.
Metapodius terminalis Dall.
Euthoctha goleator Fabr.
Anasa tristis De G.
Anasa armigera Say.
Chariesterus antennator Fabr.
Catorintha mendica Stål.
Narnia pallidicornis Stål.
Hypselonotus punctiventris Stål.
Alydus conspersus Mont.
Alydus quinquespinosus Say.
Stachyocnemus apicalis Dall.
Harmostes reflexulus Stål.
Corizus lateralis Say.
Leptocoris trivittatus Say.

Pyrrhocoridæ.

Largus sinctus.
Dysdercus suturellus Scha.
Dysdercus flavolimbatus Stål.
Dysdercus splendidus Dist.

Lygæidæ.

Peliopelta abbreviata Uhl.
Gonianotus marginepunctatus Wolff.
Œdancala dorsalis Fabr.
Myodocha serripes Oliv.
Blissus leucopterus Say.
Microtoma carbonaria Rossi.
Sphragisticus nebulosus Fall.
Ischnodemus falicus Say.
Cymus angustatus Stål.
Nysius ericæ Schill.
Perigenes fallax Heid.

Ligyrocoris diffusus Uhl.
Pamera basalis Dall.
Eremocoris ferus Say.
Ischnorhynchus didymus Zett.
Geocoris uliginosus Say.
Geocoris limbatus Stål.
Oncopeltus fascinatus Dall.
Lygæus kalmii Stål.
Lygæus bicrucis Say.

Berytidæ.
Jalysus spinosus Say.

Aradidæ.
Aradus similis Say.

Tingitidæ.
Piesma cinerea Say.
Corythucha arcuata Say.

Nabidæ.
Coriscus ferus Linn.
Coriscus pallescens Reut.

Phymatidæ.
Phymata erosa Linn.

Reduviidæ.
Melanolestes picipes H. S.
Sinea diadema Fabr.
Acholla multispinosa De G.

Emesidæ.
Emesa longipes De G.

Clinocoridæ.
Cimex lectularius Linn.

Anthocoridæ.
Triphleps insidiosus Say.

Capsidæ.
Lygus pratensis Linn.
Lopidea media Say.
Calocoris rapidus.
Hyaliodes vitipennis Say.

Notonectidæ.
Notonecta undulata Say.

Anisops platycnemis Fieb.

Nepidæ.

Ranatra quadridentata Stål.

Belostomidæ.

Benacus griseus Say.

Belostoma fluminea Say.

Veliidæ.

Hebrus americana Uhl.

Gerris marginatus Say.

Gerris regimis Say.

Functional Relation of Cæcal Bacteria to the Host Insect.

In considering the possible relation of the cæcal bacteria to the life processes of the insect, a digestive function is at once suggested on account of the great number and apparent limitation of these organisms to the digestive tract of the host. One point, however, that was rather puzzling and decidedly difficult to understand on this basis was the peculiar localization of the infection in relation to the digestive portion of the gut.

Digestive cæca are very common in other groups of insects, occurring notably in the Orthoptera, Coleoptera, and Diptera, as well as in several other orders, but with a few isolated exceptions these organs are invariably located toward the anterior end of the midgut, often serving as reservoirs for the food in which certain definite digestive processes take place. In the Heteroptera, however, the cæca, and consequently the intestinal bacteria, are located at the very posterior end of the midgut, and these organs appear to have lost their direct digestive function, since neither they nor the fourth stomach itself ever seem to contain any food, the last stages of digestion apparently taking place in the third stomach, which is usually found to contain a mass of food material in process of digestion. Just how to correlate the singular localization of the intestinal bacteria in these insects with any digestive process was by no means clear, and this remained more or less of a mystery until explained by culture experiments.

It is a well-known fact that most insects, whether feeding on solid or liquid food, support a great variety of saprophytic and

parasitic bacteria and protozoa in the alimentary canal. This relation is especially marked in those insects feeding normally on solid materials which may be more or less contaminated in the beginning, but it also applies to sucking insects which feed normally on sterile liquids, although such insects are usually less heavily infected. Mosquitoes and other blood-sucking insects are excellent examples of this, for while they feed largely on the normally sterile blood of vertebrates they usually contain a great variety of bacteria and flagellate parasites in the stomach and intestine, which clearly have no relation to the vertebrate from which the food was secured.

Early in the work a peculiar infection was observed in *Peribalus limbolarius*, a pentatomid in which the cæca are well developed. In the specimens of this species, collected from certain localities, fully ten per cent. of the individuals showed an extremely heavy infection of the large sac-like reservoirs of the salivary glands (Plate II., Fig. 4) with a flagellate of the *Herpetomonas* group. This organism was apparently going through a normal developmental cycle in these organs, as all stages could be readily observed, ranging from minute, rounded or pear-shaped, non-motile bodies, with a distinct nucleus and blepharoplast, but with no flagellum, to the long, slender, typical *Herpetomonas* stage. Multiplication rosettes were also abundant, showing that the organism had evidently fully established itself in these organs. Infection with organisms very similar to the species found in *Peribalus* have been observed and described in connection with a very large number of different insects, including especially many Diptera and predaceous Heteroptera, but so far as I have been able to discover, these infections have been confined to the stomach and intestine and are never known to be localized in the salivary glands.

In working up the development of the *Herpetomonas* from *Peribalus*, the alimentary canal was searched very carefully in a large number of individuals, as it was thought that the organism observed in the salivary glands probably represented the final stage in the life history of some intestinal form; but although a very thorough search was made, not the slightest indication was found of an invasion of the midintestine by this

parasite, although the salivary glands often appeared almost completely filled with them.

Some time later, upon examining specimens of *Podisus maculiventris*, an insect of the same family, but in which the cæca are wholly wanting, a similar infection was observed. This time, however, the alimentary canal was the seat of the infection; and although a very careful examination was made of the salivary glands in specimens showing a very heavy intestinal infection, these organs were found to be invariably free from any infection with the flagellate. In *Podisus* the infection appears to be remarkably common, fully fifty per cent. of the specimens from some localities containing this parasite. The third stomach seems to be the place of greatest multiplication of the flagellates in this insect, and not only the *Herpetomonas* but also various forms of foreign bacteria were observed in this region. This is in marked contrast to the condition found in the midgut of those forms which are provided with cæca; for of the hundreds of typical pentatomids examined, flagellates were found in the alimentary canal of but two specimens of *Cœnus delius*, which were apparently on the point of dying when dissected, and in these the parasites were confined chiefly to the first stomach instead of reaching their greatest development in the third stomach as in *Podisus*.

For a long time after the singular infection of the salivary glands in *Peribalus* had been observed, no satisfactory explanation could be offered for the strict localization of the typical intestinal flagellates in these organs. It was not realized at first that there might be some possible connection between this phenomenon and the bacteria normally infecting the cæca of the insect, but later when this case was compared with similar infections in such forms as *Podisus*, in which the cæca were wholly wanting, it seemed that the cæcal bacteria must in some way be responsible, and this view was later confirmed by direct culture experiments.

We may assume that these *Herpetomonas* parasites, upon being continually introduced into the alimentary canal of *Peribalus*, and being unable to develop in the midgut in the presence of the antagonistic cæcal bacteria, gradually become adapted to a life

in the bag-like salivary reservoirs instead of being excluded entirely as was apparently the case in most other Heteroptera in which the cæca are present.

As a result of the apparent failure in the culture work with *Murgantia* and the other Pentatomidæ followed by the successful cultivation of the cæcal bacteria from *Anasa tristis*, it became evident that at least one perfectly clear-cut function possessed by these organisms, whether of profound importance to the host insect or not, was the antagonism which they certainly show towards other bacteria and protozoan parasites which would normally be expected to occur in the intestine of such insects.

In the culture work on the Pentatomidæ it was found that the entire cæcal system of one of these insects could be removed and dropped directly into a tube of bouillon or other media, where it would remain for a month or more without a trace of growth developing. This was not an occasional occurrence, but was invariably the result secured where the Pentatomidæ were used and demonstrated conclusively that the cæcal bacteria are not only antagonistic to the ordinary saprophytic and parasitic bacteria, but prevent their development entirely, and apparently kill them when they invade the alimentary canal of these insects.

In the somewhat similar association described by Petri for the larva of *Dacus oleæ* the bacillus concerned was found to secrete an active lipolytic enzyme which presumably assisted the insect in digesting its oily food, but unfortunately Petri apparently did not consider the possible antagonism of these cæcal bacteria of the fly toward the forms which commonly invade the alimentary canal of such insects; and it is consequently impossible to say whether the cæcal bacteria of the little fly resemble those of the Heteroptera in this regard or not.

In regard to a digestive function for the cæcal bacteria of the Heteroptera, it can only be said that in cultures these bacteria apparently secrete no enzyme that could be of any very evident assistance in the digestive processes of the insect, and this agrees perfectly with the peculiar localization of these organisms at the extreme posterior end of the digestive portion of the gut, which in itself would render the probability of their assuming an impor-

tant part in the digestion of the insect extremely small, even though they had been found to secrete important digestive enzymes.

The deficiency in enzyme production shown by these organisms will be taken up in connection with the culture work, and will not be discussed here; although it might be mentioned that when the cæca, together with a considerable section of the intestine was removed, as in the Pentatomidæ, and dropped into a tube of bouillon, the tissues would remain white and apparently normal in every way for several weeks, or longer, although crammed full of the cæcal bacteria. This would show, at least, that the cæcal bacteria do not secrete a proteolytic enzyme. The same thing was also observed in cultures from *Anasa tristis*, the tissues remaining unchanged even after the cultures had been growing vigorously for a week or more.

In the case of certain of the intestinal bacteria of the higher vertebrates, there is a well-known association existing between these organisms and the host that is remarkably similar in many ways to the one just described for the Heteroptera, although as might be expected, it is complicated, in this case, by a vast number of factors that do not have to be taken into consideration in a treatment of this relation in the much more simple insects.

Practically all the higher animals harbor certain varieties of intestinal bacteria which have become so intimately associated with the host that they are generally referred to as the "normal" intestinal bacteria; and there has been a tremendous amount of work done in the attempt to determine the exact functional importance of these organisms from the standpoint of the host. As might be expected, by far the greater part of this work deals directly with those forms peculiar to man and the higher vertebrates, but as many of the principles which have been worked out for the association in these animals throw a great deal of light on conditions as they exist in the Heteroptera, the following very brief summary of the pertinent work that has been done on this subject should not be out of place.

IMPORTANCE OF THE "NORMAL" INTESTINAL BACTERIA TO THE HOST.

The fact that certain specific bacteria occur with remarkable regularity in the complex intestinal flora of the higher animals was early recognized by bacteriologists, and this knowledge naturally led up to an extended discussion concerning the true relation of these "normal" intestine bacteria to the animal harboring them.

As early as 1885 Duclaux, by a simple experiment, established the fact that the higher plants are unable, in themselves, to utilize the more complex forms of nitrogen and that they are absolutely dependent upon the action of certain classes of bacteria normally present in the soil for their supply of this essential element of plant food.

In the same year, in commenting upon the results secured by Duclaux for plants, Pasteur gives with characteristic directness his views concerning the relation of intestinal bacteria to the assimilation of higher animals. In the following well-known quotation he says:

"Souvent, dans nos causeries du laboratoire, depuis bien des années, j'ai parlé aux jeunes savants qui m'entouraient, de l'intérêt qu'il y aurait à nourrir un jeune animal (lapin, cobaye, chien, poulet), dès sa naissance, avec des matières nutritives *pures*. Par cette dernière expression, j'entends désigner des produits alimentaires qu'on priverait artificiellement et complètement des microbes communs.

"Sans rien voulair affirmer, je ne cache pas que j'entreprendrais cette étude, si j'en avais le temps, avec la pensée préconçue que la vie, dans ces conditions deviendrait impossible."

Although the above hypothesis has excited a vast amount of discussion, and although a number of very elaborate experiments have been carried out to test it, the exact relation of the "normal" intestinal bacteria to the animal harboring them is, as yet, by no means definitely settled. Thus, some authorities advance the idea that these bacteria are merely saprophytic forms which, through long association, have become adjusted to conditions in the digestive tract where they exist without exerting any important influence upon the host, unless perhaps in excep-

tional cases, when they may assume the rôle of active parasites. Others hold, on the contrary, that their presence is of very great importance in the overgrowing and destroying of the occasional invader, which if allowed to develop unchecked might seriously injure the host, and also in the inhibitory action which they exert upon the common putrefactive intestinal bacteria; while still others assert that, in addition to the function just mentioned, these bacteria not only play an important part in, but are absolutely essential to proper digestion, at least in the higher animals.

The first serious attempt to determine by actual experiment the part played by intestinal bacteria in digestion appears to have been made by Nuttall and Thierfelder in their classical experiments with guinea pigs. In these experiments the young animals were removed from the mother by Cæsarian section and transferred at once to ingeniously constructed cages where they were kept under absolutely aseptic conditions and supplied with sterile food and water. Under these conditions the authors were able to keep the animals alive for ten days, at the end of which time they were found to have increased considerably in weight. Of the four animals carried through the experiment the increase in weight was found to be 5.5, 14, 16 and 28 grams respectively.

From their data the authors conclude that intestinal bacteria are not essential to digestion and their results have been widely quoted by physiologists as showing that the intestinal bacteria are at most of only minor importance in this process.

The results of these experiments are far from being conclusive, as has frequently been pointed out. Thus, Schottelius was able to keep newborn guinea pigs alive for ten days by giving them nothing but sterile water. The same author also found that the intestinal contents of a normal ten-days-old guinea pig weighed from twelve to fifteen grams, and in this case the intestine was not completely filled; while Nuttall and Thierfelder, upon examining the pigs at the conclusion of their experiments, found that the colon and especially the cæcum were crammed full, and even greatly distended, with a brown caseous material. Schottelius insists that this constantly accumulating mass of undigested food in the intestine would much more than account for the increase in weight reported by Nuttall and Thierfelder and that,

instead of increasing in weight, their animals must have been actually starving because unable in the absence of the proper intestinal bacteria to digest the food swallowed.

Pasteur's hypothesis as to the function of intestinal bacteria was, a few years later, again tested experimentally by Schottelius, who, after carrying out a carefully planned series of experiments, arrived at conclusions decidedly different from those of Nuttall and Thierfelder.

In the experiments of Schottelius chickens were used instead of guinea pigs. The artificially incubated eggs were removed and carefully sterilized shortly before the time for hatching and placed for hatching in a specially constructed, sterilized cage, together with sufficient sterile food and water to last throughout the course of the experiment. It was found that chicks hatched and reared in this way, with the total exclusion of bacteria, showed but very little growth; and while they continued to eat ravenously, being apparently unable to satisfy their hunger, they gradually became weaker and after an apparent slight increase for the first few days invariably died, usually within twenty-five days or less after hatching.

In the control cages, which contained chicks treated in exactly the same way except for the addition of a pure culture of a colon bacillus to the food, the young animals developed normally in every way and even appeared to show slightly better growth than chicks of the same age which had been allowed to run free in the laboratory as a counter check.

These results are regarded by the author as showing conclusively that intestinal bacteria, especially of the *Bacillus coli* type, are absolutely essential to proper digestion in chickens and that they doubtless have a similar function in other animals. He summarizes as follows the precise manner in which he thinks the normal bacteria influence the host:

"1. Die Darmbakterien sind notwendig für die Ernährung der Wirbeltiere und für den Menschen;

"2. Der Nutzen der normalen Darmbakterien besteht: (*a*) in der Vorbereitung der Ingesta für die Resorption der Nahrungsstoffe; (*b*) in der Reizung der Darmwand zur Auslösung der Peristaltik; (*c*) in der Überwucherung und Vermichtung patho-

gener, in den Darm hineingelangter Bakterien; (*d*) in der Festigung des Körpers gegen pathogene Bakterien und gegen Bakteriengifte."

Similar experiments with the same end in view have been carried out by Moro and by Mme. O. Metschnikoff on newly hatched tadpoles, Moro working with *Pelobates fuscus* and Mme. Metschnikoff with *Rana temporaria*. The results secured by these two workers seem to point to some important digestive function for the intestinal bacteria, although the results in neither case appear so conclusive as those given by Schottelius for his work with chicks. In the work of Metschnikoff, for example, the nearly mature embryos were removed aseptically from the gelatinous, enveloping layer and allowed to develop in vessels of sterile water under aseptic conditions. Of forty-nine tadpoles which survived the first few days of the experiment, forty-two developed accidental contamination, and seven remained sterile throughout the experiment.

Although for some reason the mortality was much higher among the non-sterile tadpoles, and although the sterile ones actually lived longest, the development of the sterile ones was much slower than that of the non-sterile, the minimum weight and length of the non-sterile corresponding closely to the maximum of the sterile. These results, however, are regarded by the author as sufficiently conclusive to warrant the assertion that intestinal bacteria are necessary for life and development of tadpoles.

Cohendy, on the other hand, in duplicating the work of Schottelius on sterile reared chicks secured results which he regards as proving conclusively that bacteria are in no way necessary to the normal development of the chicken; and he accounts for the results of his experiments as contrasted with those of Schottelius as being due to the superior technique which he claims to have employed. In the course of this work he was able to keep chicks under strictly aseptic conditions for a maximum of forty days during which time the animals developed normally, there being no essential difference in growth and metabolism between the sterile chicks and those kept as a check, and the sterile chicks when placed under natural conditions, after

having been kept wholly free from bacteria for a considerable time, developed into perfectly normal adults. His conclusions, which are quite different from those of Schottelius, are: "La vie sans microbe est possible pour un vertébré—le poulet—pourvu normalement d'une riche flore microbienne. Cette vie aceptique n'entraîne par elle-même aucune déchéance de l'organisme."

Insects and other invertebrates have been employed by a number of investigators, as Bogdanow, Woolman, and Delcourt and Guyenot, for working out this same problem. Of these the work of Delcourt and Guyenot is especially notable, for while no vertebrate has, so far, been carried successfully through its normal life cycle in the total absence of intestinal bacteria, these authors have succeeded in keeping flies of the genus *Drosophila* for as many as twenty generations under aseptic conditions, finding that growth in the sterile cages was perfectly normal and fully as rapid as in similar but contaminated cages. It also developed that the mortality was remarkably reduced, the eggs almost all hatching from the sterile flies, while in the septic cages whole broods would frequently die.

Bogdanow and Woolman, who also worked with flies, chiefly *Lucilia cæsar* and *Calliphora vomitoria*, both succeeded in rearing these insects with the exclusion of bacteria, but their conclusions as to the importance of intestinal bacteria in growth and development are somewhat different. Bogdanow found that, while the larvæ would develop under these conditions, growth was greatly retarded, and practically all died at or before pupation although one would occasionally pupate and develop into an undersized fly. He concludes that his experiments show that bacteria, probably those species which decompose protein, are necessary to the normal development of these flies.

Woolman, however, was able to carry the larvæ through from sterile eggs to normal adults. During the first few days after hatching the growth of the sterile maggots was noticeably slower than in the septic cages, but later this difference tended to disappear, and when mature the sterile larvæ showed practically the length and weight typical for the species, pupated, and developed into perfectly normal adults. This slight check in growth shown by the maggots at first was attributed by the

author to the sterilization of the media at too high a temperature, as it was found that the difference in rate of development largely disappeared when the food was sterilized by the discontinuous method at low temperatures. Maggots hatched from sterile eggs were also treated with cultures of various bacteria, including *Bacillus coli*, *Bacillus proteus vulgaris*, *Bacillus putrificus*, and others, in order to determine the part taken by proteolytic bacteria in development, as suggested by Bogdanow, but the infected larvæ developed no more rapidly than the sterile ones and where *Bacillus putrificus* was used growth was clearly retarded and the larvæ died regularly before reaching the pupa stage. Woolman concludes that: "Cet example d'un être qui, à l'état naturel, semble vivre en association étroite avec les bactéries, montre clairement que la vie animale est possible en dehors de toute intervention des microorganismes."

It can hardly be doubted that many insects, such as those living parasitically in the body cavity of the host, normally exist during the greater part of their life cycle in the total absence of bacteria; and according to Portier this is also true of the larvæ of certain leaf-mining microlepidoptera. This author found that about 30 per cent. of the *Lithocolletis* larvæ infesting oak, elm, etc., were wholly free from bacteria and that practically 100 per cent. of the larvæ of a species of *Nepticula* infesting the rose and which do not void the excrement on the exterior of the mine were also sterile.

Notwithstanding the direct experimental evidence in support of a digestive function for intestinal bacteria, as advanced by Schottelius and others, the view appears to be generally held by physiologists, largely on account of the characteristic limitation of the different classes of intestinal bacteria to certain well-defined regions of the digestive tract, that in the higher animals at least, the actual solution of the nutrient materials in the food, in the absorptive portion of the gut, is performed chiefly if not entirely by the digestive secretion produced by the animal itself and that the chief function of the "normal" intestinal bacteria consists, not in any direct action on the food in preparing it for resorption, but rather in preventing indirectly the undue multiplication of injurious forms which are continually invading the intestine of the host.

There appears to be little question that the normal intestinal bacteria of higher animals, such as the groups represented by *Bacillus coli*, *Bacillus* (*lactis*) *aerogenes*, and *Bacillus bifidus*, do possess definite antagonistic properties for the less perfectly adapted species, although the exact mechanism of this antagonism has been explained in a number of different ways. Some would account for this phenomenon as being due largely to overcrowding, with consequent starvation of the less hardy species. The normal forms being constantly present in the intestine in large numbers, and well adapted to vigorous growth in certain well-defined regions, would naturally be better able to appropriate any nutrient materials which had escaped resorption by the host than those forms which only occasionally invade the intestine. The various products of fermentation produced by these bacteria, especially the organic acids, are also held to be of very great importance in this same connection.

Another and apparently an exceedingly important factor in this antagonism lies in the production by these organisms of definite toxin-like bodies, the "autotoxins" of Conradi and Kurpjuweit, which in some way clearly exert a restraining influence upon the development of many bacterial species.

These latter substances, which have been carefully studied especially for *Bacillus coli*, by Eijkman, as well as by Conradi and Kurpjuweit and others, are regarded by these authors as mainly responsible for the familiar weakening and ultimate death of bacteria in old cultures which was formerly held to be due chiefly to a gradual exhaustion of the nutrient materials, resulting in death by starvation.

It was found by Eijkman, and later by Conradi and Kurpjuweit, that such an old and apparently exhausted culture could again be made to support growth by inactivating the toxins, as by heating to 60° C. or by filtering through porcelain, and that these same toxins were constantly present in normal feces, where they could be detected in very high dilutions, even as great as 1–10,000.

These "autotoxins," as the name implies, affect the organism producing them as well as various other species, although, as is suggested by Conradi and Kurpjuweit, under normal conditions

in the intestine the obligate species, as *Bacillus coli*, would doubtless develop a certain amount of immunity to their own poisons which would not be shared by other transient forms.

According to Kohlbrugge there is an "autosterilization" of the small intestine in man and the higher animals generally; and normally the bacteria which occur in this part of the digestive tract are found only in the food masses, so that when the intestine is emptied the bacterial flora consequently disappears, leaving the empty intestine practically sterile. He also found that even after prolonged fasting the cæcum, that portion of the digestive tract characterized by the greatest development of bacteria of the *Bacillus coli* type, always contained quantities of this organism; and he insists that the cæcum and the vermiform appendix instead of being the useless, vestigial organs that they are commonly considered, are in reality of the greatest importance to the animal possessing them in functioning as a natural and safe culture place for a constant reserve supply of the colon bacillus.

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EXPLANATION OF PLATES.

Abbreviations.

c	cæca.
i	ileum.
*m*1	first stomach.
*m*2	second stomach.
*m*3	third stomach.
*m*4	fourth stomach.
mt	malpighian tubes.
r	rectum.
sr	salivary reservoir.
sg	salivary glands.

Plate I.

Fig. 1. *Anasa tristis*, posterior portion of alimentary canal with cæca.

Fig. 2. *Peribalus limbolarius*, posterior portion of alimentary canal with cæca

Fig. 3. *Thyrecoris unicolor*, posterior portion of alimentary canal with cæca

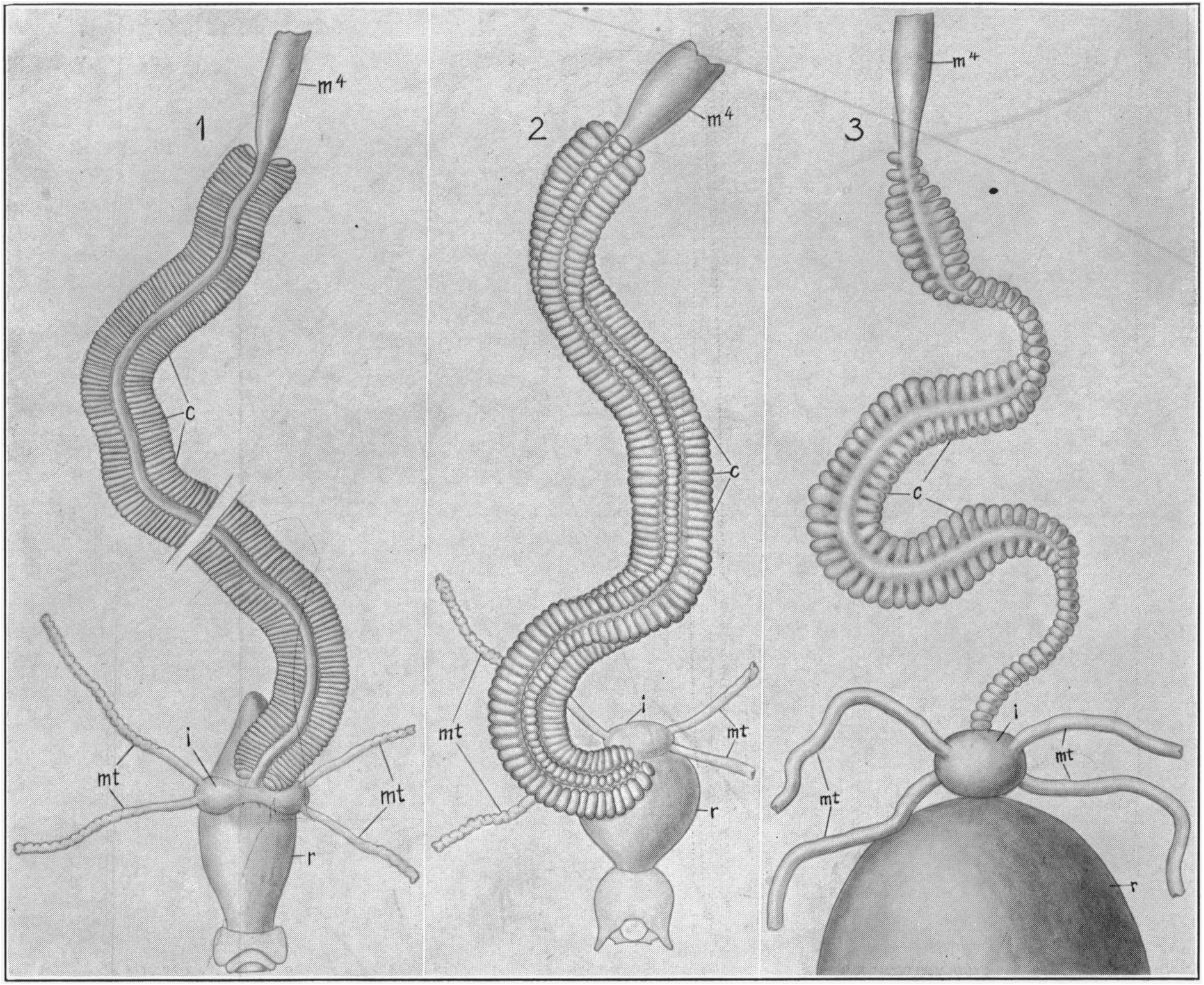

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PLATE II.

FIG. 4. *Peribalus limbolarius*, entire alimentary canal with salivary glands.

FIG. 5. *Podisus maculiventris*, entire alimentary canal.

FIG. 6. *Homoemus proteus*, entire alimentary canal.

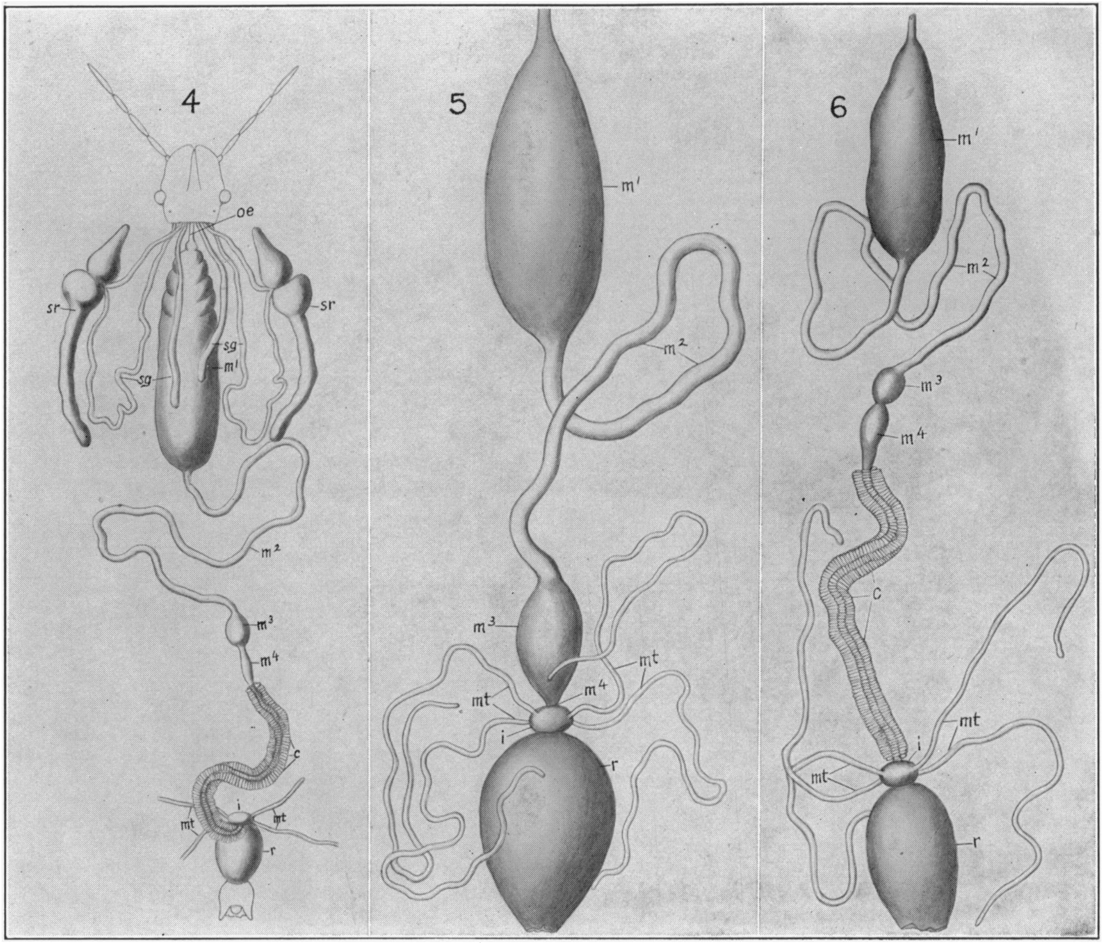
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PLATE III.

FIG. 7. *Blissus leucopterus*, entire alimentary canal.

FIG. 8. *Œdancala dorsalis*, posterior portion of alimentary canal with cæca.

FIG. 9. *Myodocha serripes*, posterior portion of alimentary canal with cæca.

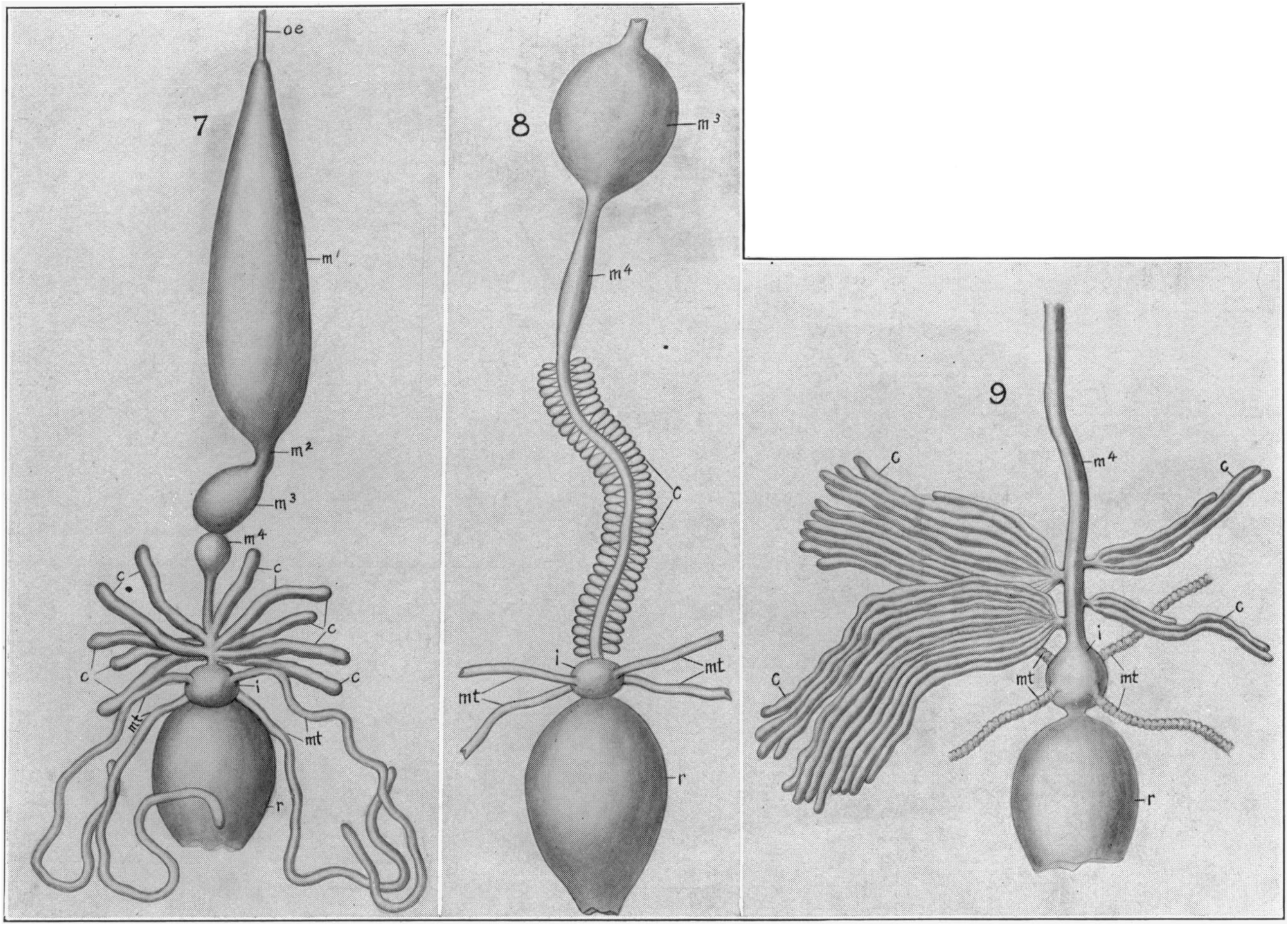

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PLATE IV.

FIG. 10. *Dysdercus suturellus*, entire alimentary canal of female.

FIG. 11. *Peliopelta abbreviata*, entire alimentary canal of male.

FIG. 12. *Peliopelta abbreviata*, entire alimentary canal of female showing pair of large accessory cæca that are not present in the male.

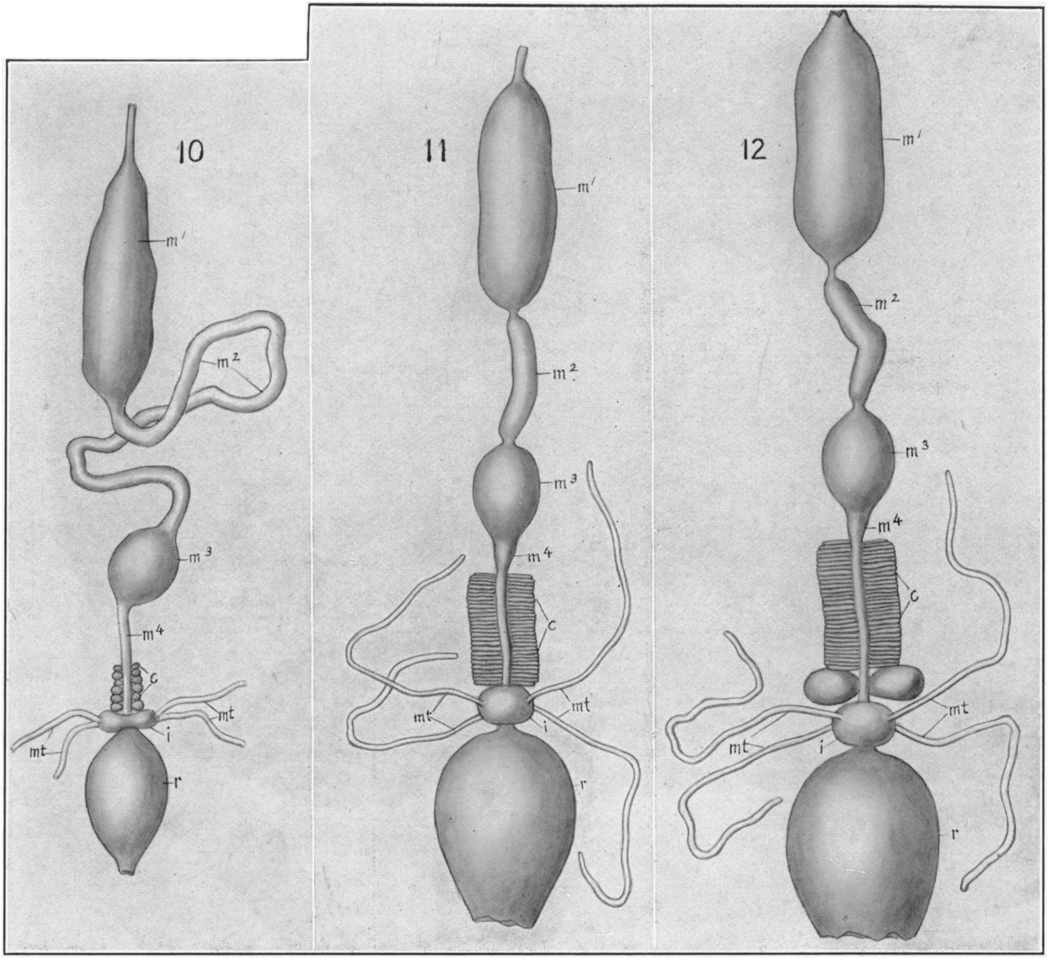

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PLATE V.

FIG. 13. *Myodocha serripes*, entire alimentary canal.
FIG. 14. *Pamera basalis*, entire alimentary canal.
FIG. 15. *Jalysus spinosus*, entire alimentary canal.

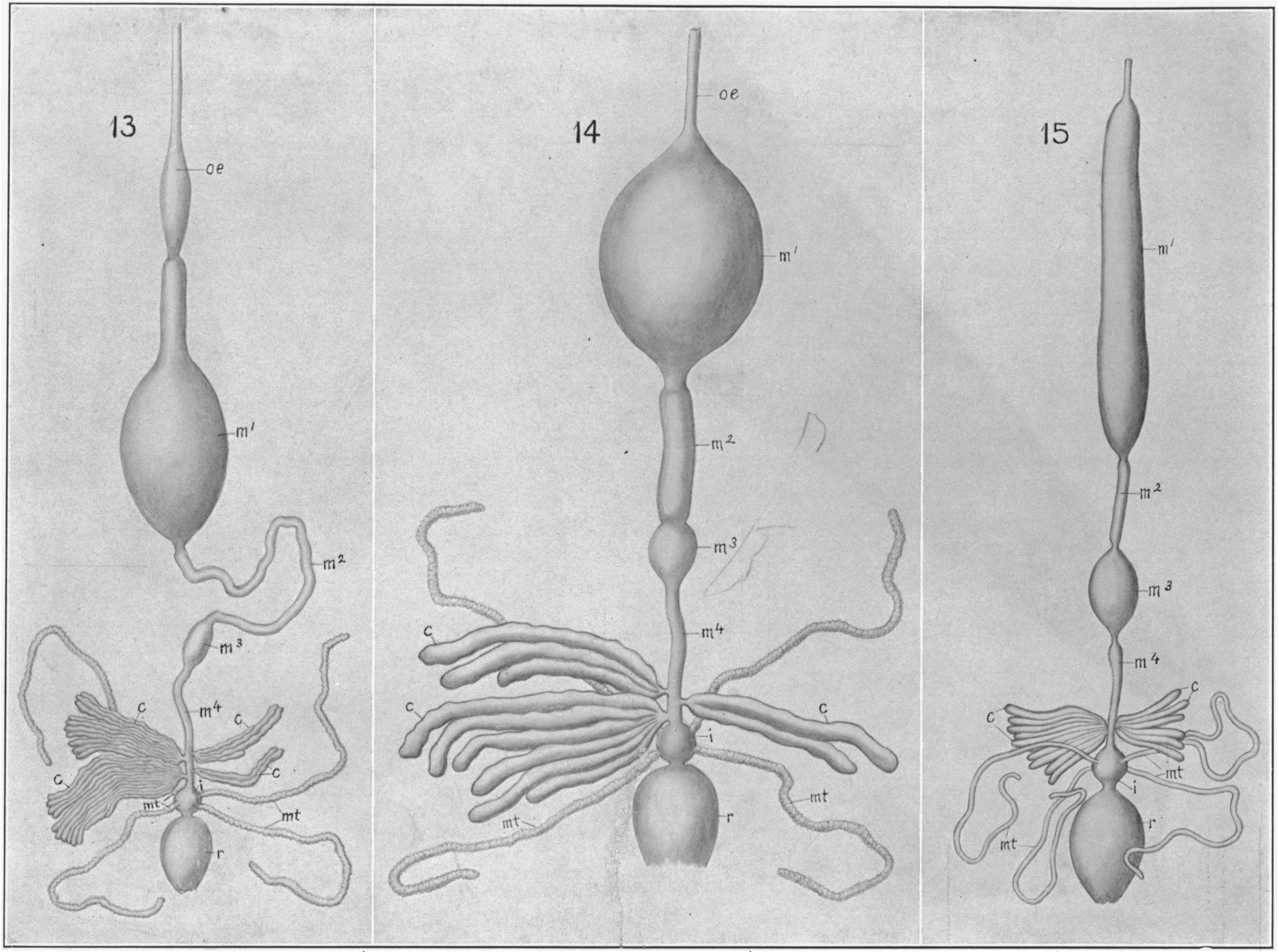
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PLATE VI.

FIG. 16. *Largus sinctus*, posterior portion of alimentary canal with cæca.

FIG. 17. *Dysdercus suturellus*, posterior portion of alimentary canal of male, showing total absence of cæca in this sex.

FIG. 18. *Dysdercus suturellus*, posterior portion of alimentary canal in female showing development of the cæca in this sex.

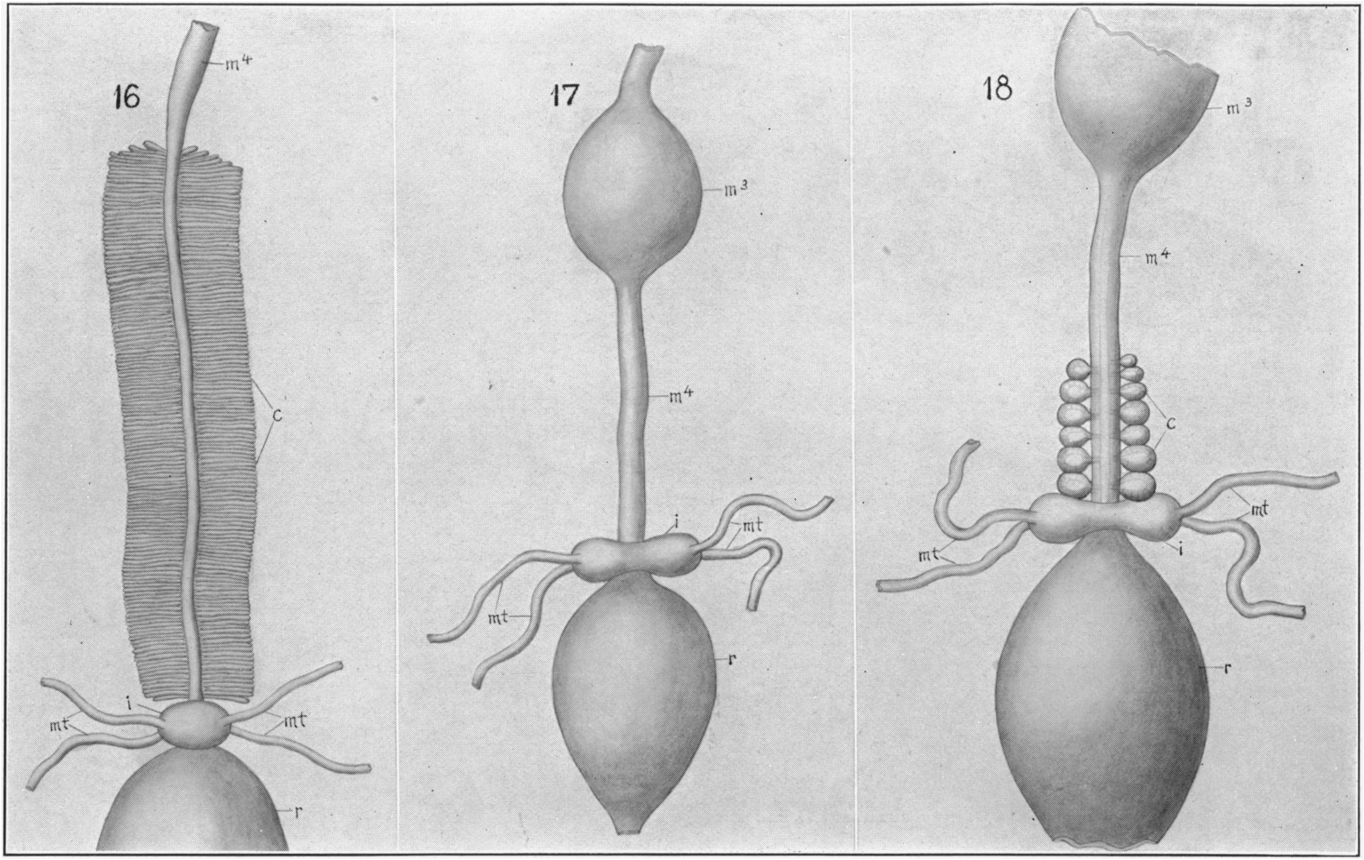

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PLATE VII.

FIG. 19. *Blissus leucopterus*, posterior portion of alimentary canal from normal insect showing the characteristic development of the cæca.

FIG. 20. *Blissus leucopterus*, posterior portion of alimentary canal from starved individual, the intestine also slightly stretched to show the grouping of these structures typical of the Blissinæ.

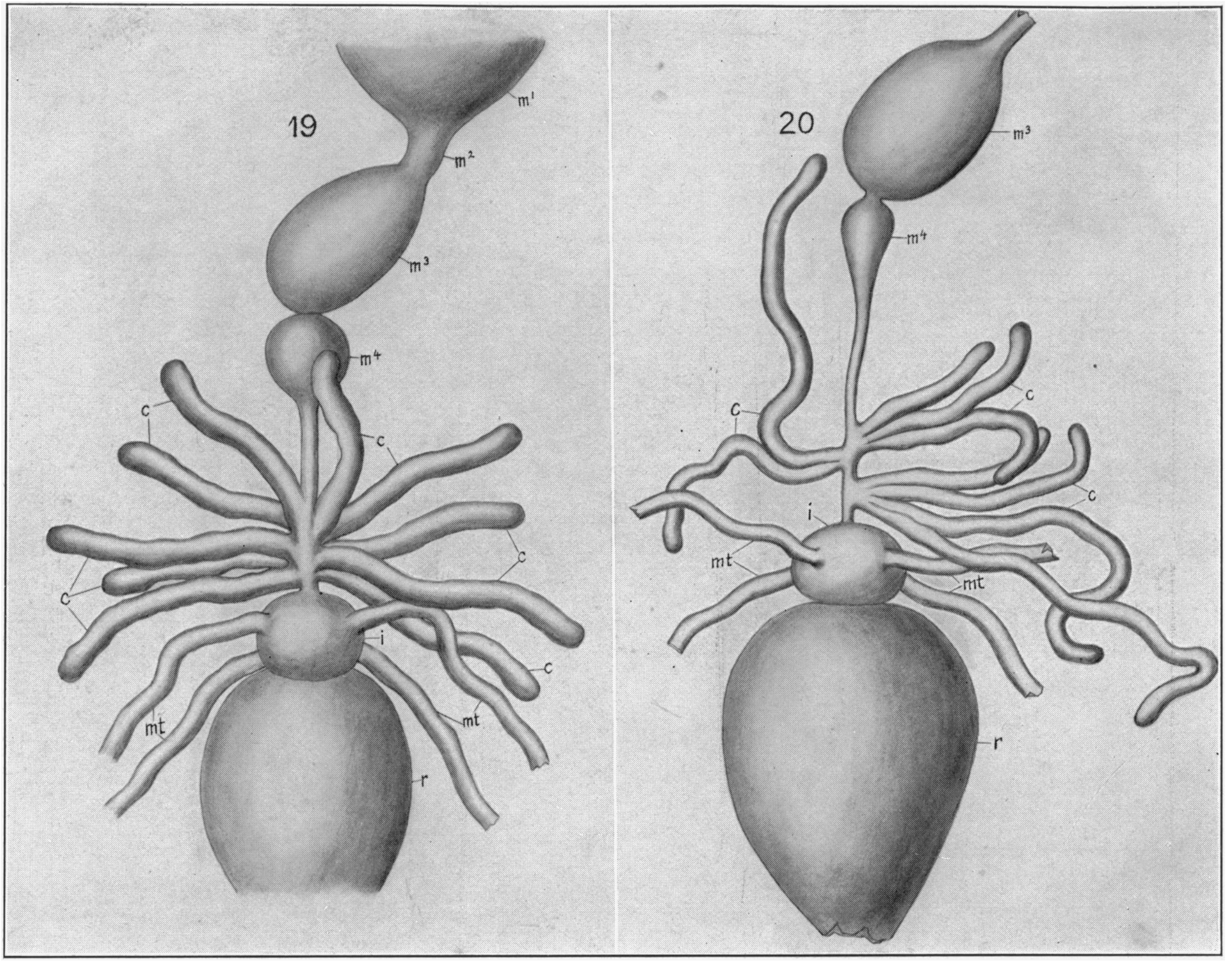

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PLATE VIII.

These drawings, with the exception of Fig. 22, were all made from methylene blue smear preparations direct from the cæca of the particular host insects given. the purpose being merely to show the relative size and form of a few of the different types of cæcal bacteria.

FIG. 21. Bacteria from cæca of *Anasa tristis*. Average size of individual rods 1 by 0.7 micron.

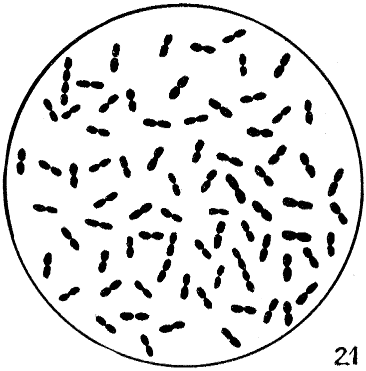
FIG. 22. Bouillon culture from cæca of *Anasa tristis*, several days old, showing common involution forms regularly produced in this medium.

FIG. 23. Bacteria from cæca of *Blissus leucopterus*. Average size of individual rods, 1 by 0.8 micron.

FIG. 24. Bacteria from cæca of *Euschistus servus*. Average size of rods, 4 by 0.9 micron, longest shown is 8 microns.

FIG. 25. Bacteria from cæca of *Peribalus limbolarius*. Size of rods, from 5 to 50 microns long by 1.2 microns.

FIG. 26. Organism from *Murgantia histrionica*. Vary from 3 to 100 microns long by 1 to 3 microns.



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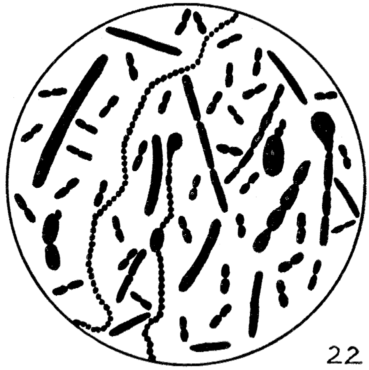

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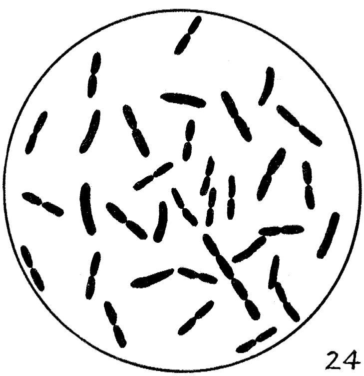

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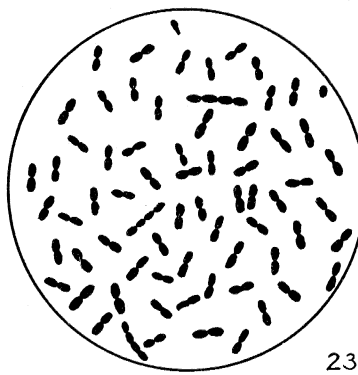

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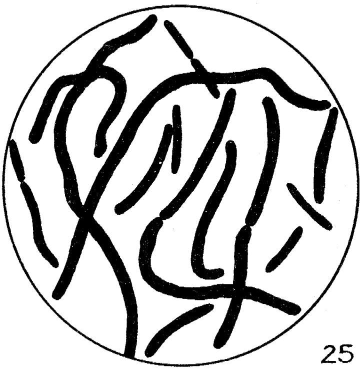

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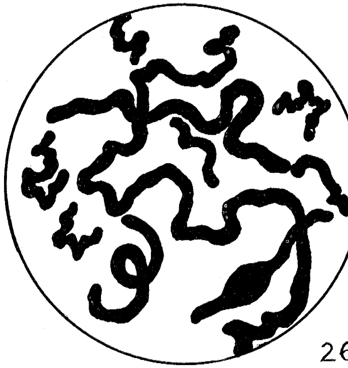

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